ENZYMES

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PREFACE

THE following lectures were given under the Herter Foundation at the University and Bellevue Hospital Medical College in the City of New York in 1910.

They were delivered before an audience of physicians and medical students and the subject was treated from the biological point of view. Here let me take occasion to express my indebtedness to the members of the faculty of the College, as well as to many other colleagues in New York, for their kindness during a visit which I shall ever remember with pleasure.

I am also indebted to Dr. W. B. Cannon for assistance which has enabled me to deliver these lectures in a language foreign to me.

OTTO COHNHEIM.

HEIDELBERG, *September*, 1910.

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INTRODUCTION

THE term "ferments" was first used early in the nineteenth century in the time of Berzelius and Schwann. Subsequently, the ferments as chemical bodies became confused with the micro-organisms which cause fermentation. To avoid confusion, Kühne gave the new name, "enzymes," to such digestive ferments as he knew—pepsin, trypsin, steapsin, and ptyalin. To-day we know that yeast, the "ferment" of the earlier authors, is a complete organism, containing enzymes like other cells and organisms, although we use the terms "ferments" and "enzymes" promiscuously.

Some hydrolytic enzymes of the yeast and of the digestive glands have been known for a long time, but interest in the enzymes has grown in recent years, especially since the great discovery of zymase by Buchner. It is now the general opinion of physiologists that living organisms effect most chemical operations by means of enzymes—*i.e.*, enzymes are the tools of the cell.

Earlier authors used for enzymes or ferments names ending in "*in*," like pepsin, trypsin, ptyalin. Recently most investigators have adopted names ending in "*ase*," which were suggested by French biologists, like maltase, lactase, protease; and they have tried to introduce a rational nomenclature, in which the name shows the action of the enzyme. The name of the chemical com-

pound which is acted upon by the enzyme is taken, and is connected with the ending "*ase*." Thus sucrase is an enzyme which acts upon sucrose or cane sugar; protease acts upon proteins; and maltase upon maltose. I think it will be impossible to employ these designations very closely, because proteins can be dissociated by different enzymes, *e.g.*, peptones by two enzymes with different qualities. Glucose is converted by zymase into carbon dioxide and alcohol, and by the enzyme of the Bacillus acidi lactici into lactic acid. Different qualities necessitate different names. I shall use the historical names, and employ the name that was given to the enzyme by its first discoverer, or that which has been generally adopted. Similarly the so-called rational nomenclature, as adopted by all chemists, employs really the old names. I shall therefore employ the terms pepsin, trypsin, erepsin, ptyalin, invertin, maltase, lactase, zymase, and steapsin. In the case of the enzyme that converts starch into dextrin and maltose, both names, diastase and maltase, are authorized because the existence of two enzymes in malt and in saliva has been suggested. The methods which enable us to obtain and to work with enzymes, have first to be dealt with, and later comes the discussion of the general properties of enzymes, after which the individual enzymes will be considered.

ENZYMES

CHAPTER I

Methods of Obtaining Enzymes

Chemistry cannot produce enzymes. They are found only as products of the living protoplasm of cells, and in all investigations on enzymes they must be separated from the cells and the organs. So far as ease of separation is concerned, a great difference exists between enzymes—the extracellular enzymes, that act outside of the cell, and the intracellular enzymes, or endo-enzymes, which in living organisms never leave the cell.

The enzymes of the first class are secreted by the glands, and in many cases these secretions can be obtained, and with them the enzymes. Human saliva is easily obtained; and following the methods of Pawlow[1] and of Bayliss and Starling[2] we can get pure saliva, pure gastric and pancreatic juice of the dog, cat, horse, goat, and other higher animals. The pure secretions of

[1] J. P. Pawlow: "Arbeit der Verdauungsdrüsen," 1898.—Nagel's "Handbuch," Bd. ii., 1906.

[2] W. M. Bayliss and E. H. Starling: Journal of Physiology, 28 and 29 (1903).

the digestive glands of some invertebrates can also be obtained. One advantage of these methods is that the product is free from many undesired substances; the chief advantage is the certainty of having the correct solubility and the proper reaction. Where the actual secretions are obtainable, investigations should never be made with extracts.

In many cases, however, the secretions cannot be obtained. For instance, the small intestine secretes a litre, or more, of fluid, but the greatest part of this enteric juice is reabsorbed before running out of a fistula, and we can get but a few cubic centimetres from a large dog, or even from the large ruminants. From small animals, most invertebrates, and micro-organisms, it is naturally impossible to get secretions in quantities sufficient for any precise investigation. We are forced to make extracts from the organs or from the whole organism, and to examine the enzymes present in such extracts. The only fluid that dissolves enzymes is water; no enzyme is extractable by strong alcohol, ether, benzene, chloroform, strong glycerin, etc. Diluted glycerin was often used by the earlier investigators (Wittich). It has the advantage that the growth of bacteria is checked by a glycerin of seventy or eighty per cent, and that the changes which enzymes undergo in watery solutions are prevented or much retarded by glycerin. Thus a solution of pepsin or of trypsin in diluted glycerin can be preserved for many months or even years, and the quantity of the dissolved enzyme can be measured better than if the ferment were

in a solid state. The enzyme, however, is not dissolved in the glycerin itself, but in the water diluting the glycerin; therefore diluted glycerin dissolves merely a small quantity of the enzyme, and the glycerin solutions of enzymes are weak solutions. If we want a solution comparable with the natural secreted juices, we must dissolve the organs in water, but we have the choice of pure water, or a salt solution, or a weak acid or alkaline solution. On account of its low osmotic pressure, distilled water destroys the cells and thus makes ferments accessible, while weak salt solutions dissolve more proteins than does pure water. For enzymes, however, I have seen no difference between water and salt solutions. Acid solutions are good for enzymes secreted in association with acid, such as pepsin, some proteolytic enzymes of the blood corpuscles and of invertebrates, and also the enzymes of yeast. The other enzymes are not destroyed by very weak acids, but some cell proteins are precipitated, and many enzymes are absorbed in these proteins and precipitated with them. I shall discuss this in treating of the purification of enzymes. If there is no special reason for the use of acids, as in the case of pepsin, perhaps the best and simplest method is to extract tissues with water, and to avoid other solvents. It is only with tissues which become acid immediately after death, as in the case of the pancreas and the muscles, when oxygen is lacking, that it may be advantageous to use a weak solution of sodium carbonate similar in its reaction to that of most living tissues. A stronger alkaline reaction endangers most

enzymes, and must be avoided. With water, or a very weak alkaline solution, it is very easy to extract all enzymes destined for secretion. I have obtained extracts of the stomach and of the pancreas containing more pepsin or trypsin than the natural juices. I have never seen peptone so rapidly split by the enteric juice as by a watery extract of the intestinal mucous membrane. The extraction is more nearly complete if the glands or the mucous membranes are finely minced and ground with sand. In this way, I have extracted from the intestinal mucous membrane all the erepsin in thirty minutes, using three portions of water. It is also very easy to extract diastase, maltase, and invertin from yeast, or the ptyalin from the salivary glands, in a short time.

More difficulty is experienced in extracting the endo-enzymes from the organs. The submaxillary gland and the liver contain the same two enzymes, diastase or ptyalin, which split starch or glycogen into maltose, and maltase, which changes maltose into glucose.

There does not seem to be any great difference between the salivary gland and the liver in the quantity of the two enzymes contained in the glands. A great deal of glycogen is lost if the enzymes of the liver are not destroyed immediately after death; *i.e.*, the liver yields much diastase and maltase. The enzymes cannot be extracted with equal ease from the salivary gland and liver.

In 1863 my father[1] obtained from the salivary gland a

[1] J. Cohnheim: Virchow's Archiv, 28, 241 (1863).

solution splitting starch in one minute; and using the same method, he obtained from the liver a solution splitting starch in two hours. Forty years later, Borchardt [1] had no better results when isolating the ptyalin of the liver. Neither from yeast nor from the muscles can the glycolytic enzymes be extracted without complete disintegration of cells and cell structure. For fifty years biologists debated whether the fermentation of sugar by yeast was an enzymotic process, or whether only the living yeast could burn sugar. This was decided when E. and H. Buchner and Hahn [2] were able to isolate the zymase from the cell after thoroughly destroying the cell membranes. The chemical means for dissolving out substances like glycogen from the tissues, using strong sodium hydroxide or other strong alkalies, cannot be used, because they destroy the enzymes. The disintegration must be made by mechanical means. Buchner and many others have ground micro-organisms and tissues with sand and siliceous earth, and subjected the mass to considerable pressure in a hydraulic press. Macfadyen and Rowland have frozen yeast at a temperature of $-180°$ C., and ground the solid ice without any addition. For muscles, glands, and other tissues, the best method is Kossel's.[3] The tissues or the whole animal are frozen by solid carbon dioxide, or by a salt-and-ice freezing mix-

[1] L. Borchardt: Pflüger's Archiv, 100, 259 (1903).

[2] E. Buchner, H. Buchner, and M. Hahn: "Die Zymasegärung," Munich, 1903.

[3] Berichte d. d. chem. Ges., 33 (1900).

ture, and cut by rotating knives. In this manner a snow is obtained which has no visible microscopical structure; and this is mixed with siliceous earth and subjected to the high and slowly increasing pressure of a hydraulic press. Using this method, I have obtained 60 cc. of a clear liquid from 100 grammes of fresh muscles of the cat or ox, and 40 cc. of liquid from 100 grammes of liver, spleen, or kidney. This liquid contains a fair quantity of the intracellular enzymes, but only a small amount of the cell proteins. There is no certainty that we have in solution all the enzymes existing in the cells or organs. Recently Pringsheim and Zemplén,[1] in the laboratory of E. Fischer, tried to extract the sucroclastic enzymes from some bacteria, and succeeded in many cases, but not in all, although all investigated bacteria contain the enzymes. The enzymes are retained by the proteins, while the other insoluble substances remain with the siliceous earth.

We may now proceed to discuss the difficult and important question of adsorption, or attachment of enzymes to proteins and other substances. In many cases we can overcome the adsorption and render the enzymes soluble, if we change the reaction of the pulp before expressing it. Many enzymes resemble the nucleoproteins in solubility, and are precipitated by very weak acids. These enzymes, especially, that convert disaccharides into monosaccharides, as lactase, maltase, and invertin, are fixed in, and retained by, the solids of the tissues. Another method of

[1] "Studien über die Polysaccharide spaltenden Fermente in Pilzpresssaften"; Zeitschr. f. physiolog. Chemie, 62 (1909).

disintegrating cells or tissues is by drying them. Kühne treated fresh pancreas with alcohol and ether in great excess; the well-dried powder contained all the enzymes of the gland. The dried pancreas powder was used also by Malloizel in his important investigations on the synthesis of fats induced by the enzyme steapsin. Buchner placed yeast in an excess of acetone and thus obtained the "dauerhefe," *i.e.*, a dry powder containing zymase. Wiechowski dissolved the fats and lipoids of the fresh liver in toluene, and dried the undissolved residue at 50° C. The enzymes can be preserved for a long time as dry powder, but they lose their solubility. The "dauerhefe" when added to a sugar solution ferments the sugar, but it is not possible to extract the zymase from this preparation. It is generally necessary to purify the enzymes and this is done by removing contaminating substances from the fresh extracts and then evaporating the pure solution to dryness. The pepsin and trypsin of commerce are dry powders prepared in this way.

Between the endo-enzymes working within the protoplasm and the enzymes destined for secretion, are the enzymes of the mucous membranes of the stomach and of the intestine in which the chyme is absorbed. The chyme passes through the cells, but becomes no part of the protoplasm. It has been observed that the enzymes formed by the cells of the small intestine—the erepsin, the invertin, the maltase, and especially the lactase—are to be found in very large quantity in the extract of the mucous membrane, but in small quantity in the secretion

or in the contents of the intestine. It seems to me that the simplest explanation of this fact is that the peptones and the disaccharides are converted to a small extent in the lumen of the intestine, and to a greater extent while they pass through the cells. For the stomach this explanation seems certain. Tobler,[1] when investigating the digestion in the stomach, using a good quantitative method, found that twenty to thirty per cent of the proteins of fresh meat are absorbed in the stomach, particularly in the right side of the stomach in the antrum pylori. Bergmann[2] has discovered a protease or an erepsin which splits peptone into amino-acids, in the mucous membrane of the antrum pylori, but this protease is not present in the chyme ejected through the pylorus; at least I could find no traces of it.[3] The function of this strong enzyme is to attack peptone passing through the cells of the absorbing membrane. These enzymes—the protease of the stomach and the erepsin and the lactase of the intestine—work within the cell, but they can be as easily extracted as those enzymes which are secreted.

Some authors have believed that they could investigate tissue enzymes without dissolving the tissues. Salkowski,[4] Jacoby,[5] Hedin and Rowland,[6] and others have placed

[1] L. Tobler: Zeitschr. f. physiolog. Chemie, 45 (1905).

[2] P. Bergmann: Skandinav. Archiv f. Physiol., 18 (1906).

[3] Cohnheim: Münchener med. Wochenschrift, 1907.

[4] E. Salkowski: Zeitschr. f. klin. Medicin, 17 (Suppl.), 1891.

[5] M. Jacoby: Zeitschr. f. physiolog Chemie, 30 (1910).

[6] S. G. Hedin and S. Rowland: Zeitschr. f. physiolog. Chemie, 32 (1901).—O. Schumm, Hofmeister's Beiträge, 7 (1905).

finely minced liver in water saturated with chloroform or toluene, and allowed the enzymes of the dead cells to act on the substances surrounding them.

For some time I followed the same method in investigating the glycolytic enzymes of muscles. I placed the finely minced muscles in water containing glucose and the activator of the enzyme extracted from the pancreas. We can detect and estimate in this manner unstable enzymes which are destroyed whilst being extracted, but we lose the advantage of limiting the action to selected substances.

Many proteolytic or so-called autolytic enzymes of the liver, spleen, and kidney can be extracted without difficulty in the same manner as erepsin from the mucous membranes; and with the exception of this extract, we know only of the existence in these organs of some proteolytic enzymes. We can find, for instance, in the liver three classes of proteolytic enzymes of different characters and different biological importance. First, enzymes derived from the blood left in the vessels of the liver. These interesting enzymes, which Opie[1] has investigated so thoroughly, will be dealt with later. Secondly, there is erepsin, destined to act upon the peptones which have passed the intestinal wall unchanged and which circulate in the blood. Thirdly, there may be an enzyme which attacks the proteins of the living protoplasm, and thus in

[1] E. L. Opie: Rockefeller Institute for Medical Research, 4 (1905); 6 (1906); 8 (1908).

metabolism draws upon the real body of the cells. Apparently there would be a great difference in metabolism whether the enzyme attacked absorbed protein or the protein of the cell itself. If we extract the enzyme, we can distinguish between erepsin and trypsin, because trypsin leaves untouched the true albumins and globulins of the protoplasm, while erepsin slowly attacks the histone of the nucleus. The strong proteolytic enzyme yielded by the so-called liver of molluscs nearly resembles erepsin. Vernon and Hedin [1] extracted and isolated the enzymes and demonstrated their resemblance to erepsin. The study of the enzymes within the tissue, and without extracting them, cannot be even approximately complete; we must always try to extract the enzymes.

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[1] Vernon: Journ. of Physiol., 30 and 33.

CHAPTER II

The Purification of Enzymes

For many purposes we can use, without further treatment, the extracts of the tissues containing ferments. The investigations of zymase by Buchner[1] and his collaborators were made with the press-juice of the yeast. Emil Fischer has studied the enzymes by hydrolyzing the disaccharides with the simple, unpurified extracts of yeasts and animal tissues. For the glycolytic enzymes in the tissues of the higher animals, no method is known to-day by which the enzymes may be purified without destroying them. Other enzymes withstand chemical treatment, and in many investigations it is necessary to remove proteins, nucleic acids, salts, carbohydrates, etc., accompanying the enzymes in the watery extract. Even the natural secreted juices are not pure solutions of enzymes. The saliva and the enteric juice contain sodium chloride and sodium carbonate; the pancreatic juice and the secretion of the liver of the cephalopods contain sodium carbonate and perhaps carbonates of organic bases; the gastric juice contains hydrochloric acid; and, in addition, all these juices contain proteins and nucleic acids, and perhaps also lecithins. The organism can secrete liquids

[1] E. and H. Buchner: "Die Zymasegärung," Munich, 1903.—E. Buchner and his collaborators: Ber. d. deutsch. chem. Ges., 1896–1908.

which do not contain proteins or nucleic acids, *e.g.*, urine, bile, sweat; or liquids which contain these bodies in traces, *e.g.*, the aqueous humor, the cerebrospinal fluid, and some liquids in invertebrates, for instance echinodermata. But these secretions are free from enzymes. The secretions containing enzymes are rich in proteins and nucleic acids. The quantity of these in the gastric and pancreatic juice varies proportionately with the enzymes, and their solubility is like that of the enzymes. Therefore many authors have thought them to be the enzymes; but they are mistaken, because we can separate the enzymes from the proteins accompanying them in extracts and in secretions. I shall discuss their chemical nature later on.

In order to purify substances chemically, we precipitate either the substance or the impurities. Now we do not know any reagent which will bring down enzymes in a specific manner, but the latter have the property of being adsorbed by finely divided solids, and especially by precipitates during their formation in a solution. We have to consider three possibilities, although practically it is not easy to tell which one we are dealing with in a special case.

1. Enzymes and other substances are similar to one another in solubility and precipitability. Thus all proteins occurring in animal or vegetable bodies are salted out by ammonium sulphate, and, so far as we know, so are all enzymes.

2. Enzymes are brought down by physical adsorption,

by precipitates forming in an enzyme solution; for instance, by the precipitate of calcium phosphate formed on adding lime-water and phosphoric acid, or the precipitate of cholesterin formed on adding an alcoholic solution of cholesterin, to the aqueous solution of enzymes. Proteins, animal charcoal, and siliceous earth also bind enzymes by physical adsorption.

3. Enzymes are fixed and held by solid substances in chemical connection, and in this manner they are attached to many proteins, in addition to the mere physical adsorption, which occurs in the cases of animal charcoal and siliceous earth. Physical adsorption precipitates all enzymes, while the chemical connection is a specific one; but the connection is apparently a loose one which can be broken by hydrolysis. The completeness of hydrolytic dissociation depends on the amount of water, and we can thus separate enzymes and proteins, or enzymes and animal charcoal, by the addition of an excess of water; we can wash out the enzymes from the solids, though they are chemically connected with them.

The chemical connection, the physical adsorption, and the precipitability of enzymes in the presence or absence of proteins, are available for the purification and separation of enzymes chiefly by three methods:

1. We can precipitate both enzymes and proteins with alcohol (Vernon[1]), with ammonium sulphate (Kühne[2]

[1] H. M. Vernon: Journ. of Physiology, 30 (1903).
[2] W. Kühne: Naturhist. Med. Verein, Heidelberg, 3 (1886).

and others), with uranyl acetate (Jacoby[1]—the aldehydase of the liver; Roselle[2]—trypsin), or with phosphoric acid and lime-water (Brücke and others—gastric juice and saliva). We allow the precipitates to stand for some time, until the proteins become insoluble, and then dissolve out the enzyme with water, or in the case of pepsin, with hydrochloric acid. Using the phosphoric acid and lime-water method, Brücke isolated pepsin, though with great loss, as the first enzyme to be obtained at all free from proteins; my father employed the same method for ptyalin, which was not destroyed by this treatment. So with ammonium sulphate the erepsin of the intestinal mucous membrane was separated from a great mass of the proteins and other impurities. The ammonium sulphate and the other crystalloids were subsequently removed by dialysis, leaving a solution rich in ferments but poor in all other substances.

2. We can split the proteins wholly into amino-acids or peptones, and then precipitate the enzyme with ammonium sulphate. By this method Kühne obtained nearly pure trypsin. This method is used for pepsin and for the enzyme oxidizing aldehydes in the liver, but cannot be applied to most enzymes which are destroyed by strong proteolytic ferments. Even trypsin itself, purified in this manner, loses a great deal of its power.

3. We can precipitate many proteins, nucleic acids, and other substances, by very dilute acetic acid, while the

[1] M. Jacoby: Zeitschr. f. physiol. Chemie, 3 (1900).
[2] Roselle: Diss. Strassburg, 1901.

enzyme remains in solution. In such manner Kossel and Dakin[1] freed liver arginase from most proteins. Essentially the same principle applies when fresh tissue-extracts are allowed to stand for some time; coagulation being then due to the weak acid reaction resulting in muscles and other tissues shortly after death. If we dialyze the extract at this time in running water, we remove both the proteins which become insoluble and the salts and other crystalloid substances which pass through the parchment. After the dialysis, or after standing, we filter off the precipitate. As a rule it is useless to filter under pressure through a Chamberland or similar filter.

Nevertheless, the advantage of Buchner's method consists not only in the complete disintegration of tissues and structures, but likewise in the filtration of the press-juice through siliceous earth, which retains the nucleoproteins, the globulins, and all proteins coagulating spontaneously. Almost the only proteins found in the press-juice of the muscles and the liver are albumin and the hæmoglobin of the blood. These juices are in most cases very rich in enzymes.

Using one of these methods it is easy to obtain proteolytic enzymes and steapsins, but many sucroclastic enzymes are retained by the proteins, and cannot be dissolved out again by water. Sometimes it is possible to dissolve them by very weak alkali, but the necessary conditions have not been sufficiently studied.

[1] A. Kossel and H. D. Dakin: Zeitschr. f. physiol. Chemie, 41, 42 (1904).

If we do not succeed in bringing the enzymes into solution, we must add the solid precipitate to the solution containing carbohydrates or fats or proteins, which are to be acted upon by the enzyme. We do not know whether the enzyme works under such conditions in a solid state and converts carbohydrates and other substances only by contact, or whether the enzyme passes into solution. We shall see that we must assume a chemical connection and combination between enzyme and substance. Perhaps the chemical compound thus newly formed is more soluble than the free enzyme. This would be a good explanation of the action, for instance, by which the mucous membrane of the small intestine converts milk sugar, although it is impossible to find lactase in the extracts, or the ability of dried pancreas powder to dissolve protein, which is lacking in extracts made from this powder. We must always remember, however, that the difference can be produced also by an improper reaction of the extract. It is an important fact, however, that we can use the dry powder for many purposes.

With these methods we can easily remove proteins and other harmless, indifferent substances. The chief difficulties which complicate the purification, extraction, and investigation of enzymes, are the presence of other enzymes, and the anchorage of the enzymes in the cells. The avoidance of these difficulties is the chief advantage of the secreted natural juices as compared with the extracts.

1. By no method can one enzyme be separated from

another without great loss. The gastric juice contains only the well-known proteolytic enzyme, pepsin, which dissolves natural proteins and converts them into peptones; but the process never goes beyond the stage of yielding biuret-giving substances. The mucous membrane of the stomach, and therefore its extracts, contains, besides pepsin destined for secretion, an erepsin acting intracellularly and splitting peptones into amino-acids. The pepsin and the erepsin have the same solubilities, and if we free the proteolytic enzyme from impurities, we concentrate both enzymes, getting an artificial juice with properties other than those of the natural one. The contradictions of authors on the question of trypsin and trypsinogen is believed by Bayliss [1] to be susceptible of the same explanation.

2. If we microscopically study the pancreas or a salivary gland at rest, we see the cell crowded with small, refractive granules forming a mass that leaves only a very narrow clear space next to the basement membrane. In the salivary gland the cell can be wholly studded with the granular mass. During glandular activity the granules become much fewer in number, and retract to an inner narrow margin; in the salivary gland they may even disappear. There can be no doubt that during the act of secretion, the granules are discharged to form part of the secretion. They become the solid matter of the juices; therefore they must be soluble in water. If we extract

[1] W. M. Bayliss: Archiv. des Sciences Biol. de St. Pétersbourg, 11 (1 Suppl.), 1901.

the pancreas or the parotid gland while studded with these granules, they, and with them the enzymes, are dissolved immediately. As has been pointed out by Pekelharing,[1] we find in the extracts of the gastric mucous membrane the most characteristic chemical compound of the gastric juice.

There is something else to be noticed in dealing with the intracellular enzymes that never leave the cell. In the state of activity, both these enzymes and the substances hydrolyzed or oxidized by them must be in solution, for "corpora non agunt nisi soluta." To avoid the action of enzymes at rest, the two bodies must be separated from each other; they must be fixed outside the real fluid protoplasm. We know another substance which, in this respect, is analogous to the endo-enzymes; glycogen, which is soluble in water, but which may be stored in insoluble form within the cell. Ehrlich[2] found that glycogen is fixed in the liver cell by a "Trägersubstanz," a holding-substance, and that one of the color reactions attributed to glycogen is due to this substance.

Glycogen and this Trägersubstanz are rather firmly bound together. We know with what difficulty the glycogen is completely dissolved out of the organs, and with what difficulty it is freed from the last traces of impurities. It seems to me that we must also assume such a holding-substance for the intracellular enzymes which restrains the action of the enzyme even after the almost

[1] Pekelharing: Zeitschr. f. physiol. Chemie, 22 (1896); 38 (1902).

[2] P. Ehrlich: in a paper in Frerich's Zeitschr. f. klin. Med., 6 (1883).

complete disintegration of the tissues. I have cut frozen muscles using Kossel's[1] rotating knives, and I have studied the glycolytic enzyme of this snow; I have also compared the snow simply dissolved and floating in water, with the press-juice I obtained by mixing the snow with siliceous earth and pressing it. The glycolytic power of the press-juice was much greater, I think, because the siliceous earth had retained with the other proteins the Trägersubstanz of the enzyme.

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[1] Kossel: Zeitschr. f. physiol. Chemie, 33 (1901).

CHAPTER III

The General Properties of Enzymes

All enzymes are colloids, like the native proteins of the seralbumin type. Like these, they cannot be dialyzed through parchment, and are irrevocably changed on heating in aqueous solution to 60° to 70° C. The albumin is coagulated by heat, and this coagulum cannot be dissolved without conversion into peptones; the enzymes, however, disappear entirely on heating. It is possible that they are coagulated and become insoluble; it is also possible that they are chemically changed and decomposed. The only sign of the presence of enzymes is their action, and we see that the hydrolyzing or oxidizing power of a solution is lost by heating, and cannot be restored. Pfeffer was able to show that the enzymes do not volatilize with the vapor of water and thus do not leave the solution.

All enzymes are destroyed by heat. Most of them are more or less slowly impaired at a low temperature—37° to 40° C.—and many undergo a slow loss of power, at room temperature, and even at zero. Zymase and the glycolytic enzyme of muscles are destroyed in the frozen state in two to four days.[1] Enzymes are also broken down by many chemical processes, by strong acids and

[1] L. Iwanoff: Zeitschr. f. physiol. Chemie, 42 (1904).

alkalies, by alcohol, etc. Pepsin loses its enzymotic properties by contact with the weakest alkali, and many oxidizing enzymes by acid. It is possible that this alteration is the same as that which takes place at a slower rate on heating. Bayliss[1] supposes that the alteration at low temperature is reversible. He cites as an example, that toxins may become inactive toxoids while retaining their chemical character. This supposition was recently supported in a very remarkable manner by Pawlow.[2] It has always been believed that the pepsin is completely destroyed by alkali; Pawlow and Tichomirow demonstrated that this is the case only if the alkaline solution is immediately acidified; the pepsin cannot then be detected. If, however, the gastric juice be made alkaline, then neutralized, and allowed to stand for some time before acidulating it, the pepsin does not disappear, but acts almost as strongly as before. This is the first case of restitution of an enzyme after having lost its vigor. Meltzer has pointed out that this slow loss of power is greatly accelerated on shaking solutions of enzymes. The destruction through shaking takes place even at low temperatures, but proceeds more rapidly at the temperature of the body.

To-day every investigation of enzymes is retarded by the same difficulties as those attending the chemical study of proteins. The true proteins are like enzymes so far as

[1] W. M. Bayliss: Archiv. des Sciences Biologiques de St. Pétersbourg, 11 (Suppl. Pawlow-Volume) (1904).

[2] N. P. Tichomirow: Zeitschr. f. physiolog. Chemie, 55 (1908).

sensitiveness toward temperature and toward acid and alkali are concerned. Knowledge of the chemistry of the proteins stagnated for a long time, because the methods of dealing with colloids were undeveloped. It made very rapid progress as soon as it was found possible to work with strong hydrochloric acid, barium hydroxide, and phosphotungstic acid. I have cited the methods of purifying enzymes, salting out, and precipitating by weak acid or alcohol, avoiding heat. By such methods we are unable to separate compounds which can be chemically defined. It is to be hoped that Pawlow's observation may become the beginning of a really chemical exploration of enzymes.

We cannot detect or estimate the enzymes themselves. We can only observe their action. For example, we can estimate the quantity of cane sugar before and after the action of invertin; we can also determine the quantity of glucose and levulose, the products of the decomposition of the cane sugar. Further, we can estimate the quantity of the coagulable proteins before the action, and after five, ten, and fifteen minutes, or the quantity of the non-coagulable peptone; but we cannot directly estimate the invertin or pepsin. Therefore everything that disturbs the action of the enzyme seems to be a diminution, while everything that improves the action seems to be an increase, of the *quantity* of the enzyme, although possibly only due to differences in the conditions of its action.

Under these circumstances it seems to me impossible to speak of the physical properties of enzymes. It is a

rule, generally acknowledged in chemistry, that for determining the physical properties of a substance—the molecular weight, the melting-point, the osmotic pressure, the rotatory power, the electrical conductivity, the ionization—the substance must be wholly purified. We know that in enyzme solutions the weight of enzymes is much smaller than the weight of the impurities; it is clear that in such a solution we cannot define the law of action of these bodies. If we read the books or papers written on enzymes by chemists, we find that the law of Schultz states that the action of a solution of an enzyme increases, not in proportion to the quantity, but to the square root of the quantity of the contained enzyme; and the question is also debated, whether the enzyme is consumed during its action or not, etc. The prevailing great interest in the enzymes has led authors to bring up questions which nobody can answer at the present time. All we can do at present is to describe a number of the properties of enzymes which have been observed.

1. Enzymes are colloids, and do not dialyze through parchment.

2. Enzymes have an optimum temperature of action. It is well known that all chemical processes go faster with increasing temperature, and van 't Hoff enunciated the rule that the rapidity of most chemical processes is doubled or trebled when the temperature is raised 10° C. The enzymes follow this rule, but only between 0° and 40° C.; above 40° C., this power decreases rapidly. If we graphically represent the behavior of the decomposition of cane

sugar by hydrochloric acid and by invertin, with increasing temperature, we find for the acid a curve like this  , and for the enzyme a curve  , *i.e.*, a curve with a distinct maximum. It is possible that two processes are involved: the general increasing reaction with rise of temperature, and the destruction of the enzymes by heat. But this is not certain. In some enzymes we can see a slow acceleration of action at from 10° to 30° C., and a much more rapid acceleration at from 30° to 37° C. The curve has a form difficult to explain as the summation of opposed effects. It is possible that we have, besides the general acceleration with rising temperature, a special adaptation of the enzymes to the body-temperature of the higher animals. The optimum of most of the enzymes examined lies between 30° and 40° C. But we find old statements that the optimum of the diastase of yeast lies at 50° C., or that the pepsin of fish has an optimum between 2° and 5° C. These statements do not hold good, as the tissues of cold-blooded animals contain proteins coagulating spontaneously at 30° to 40° C.; if these proteins are precipitated, pepsin is precipitated by adsorption, and the extract becomes accordingly poorer without change of the pepsin itself. In the case of yeast, the better solubility at higher temperatures can explain the observation. Until we can work with pure enzymes, we shall have great difficulties in distinguishing a change in solubility from a true change in the enzyme.

3. Enzymes and Reaction. Most enzymes are exactly adapted to the reaction of their "milieu"—that is to say,

of the solution in which they occur in nature. Thus pepsin acts best in combination with acid, with hydrogen-ions. It does not digest protein, but only the chloride of the protein, and the products of the enzyme are the chlorides of peptones. The quantity of acid which is bound on the proteins and peptones, and which changes the free compounds into chlorides, is not sufficient for the action of pepsin. This was explained by Leo.[1] Pepsin needs an excess of acid for its action, that is to say, it needs free hydrogen-ions. When we study dissolved protein, we cannot see this, because the hydrochloric-proteins split off acid by *hydrolysis*. But if we use a solid protein, for instance fibrin, we find that we must add so much hydrochloric acid, that the water, in which the swollen fibrin lies, still contains acid. The natural gastric juice of the dog or of man contains one-half or more per cent of hydrochloric acid, but the juice is diluted by food to 0.35 per cent; it is then 1/10 normal hydrochloric acid, and it has been pointed out by Brücke[2] and Pawlow, that the optimum of pepsin lies at this lower concentration. The hydrochloric acid can be replaced by another acid according to the available hydrogen-ions, but it seems that the hydrochloric acid has an exceptional value, which is greater than that of other acids of the same concentration and ionization.

The necessity of co-operation between pepsin and

[1] H. Leo: Zeitschr. f. physiolog. Chemie, 46 (1905).—"Die Salzsäure-Therapie," Berlin, 1908.

[2] E. Brücke: Wiener Akad., Math.-Naturw. Klasse, 37 (1859).

hydrochloric acid has caused many errors in the chemistry of enzymes. The peptones produced by pepsin are basic, and neutralize the acids; the hydrochloric-peptones are hydrolyzed salts, and the quantity of acid set free by hydrolysis depends upon the concentration and upon the presence of other neutral salts. All these influences appear to us as a diminution or an increase of pepsin. Furthermore, we know two proteins, the myosin of muscles or syntonin, and the fibrin of the blood, which swell in acids and can be attacked by pepsin only in the swollen state. Sodium chloride and other neutral salts check this swelling, and appear as opposed to pepsin; as "antiferments." Pepsin is least suitable as an example for pointing out the laws of enzymotic action. Unfortunately, however, it is used most frequently. The relations of trypsin to the reaction have often been investigated by earlier authors, but they did not always distinguish between the speed of action of the dissolved trypsin and the ease of dissolving trypsin from the gland. The enzyme has two actions: it dissolves the protein, and it splits up proteins and peptones into simpler products. It also seems to have two different optima. It dissolves proteins when the reaction is distinctly alkaline; and it decomposes peptones best at the approximately neutral or slightly alkaline reaction which prevails in the intestine. This nearly neutral reaction is best also for the enzymes of the saliva, the intestine, and the tissues. For a long time there has been a division of opinion about the reaction of the contents of the intestine and the tissues.

The dispute arose solely because of the deficient knowledge at the time of the qualities of the indicators used in the study of the reaction. Now we know that the reaction of both tissues and contents of the intestine resembles that of a weak solution of alkali saturated with an excess of carbonic acid. Such a solution gives the optimum for the enzymes of the saliva and intestine, and for the oxidizing enzymes of the tissues. The tissue enzymes are destroyed by an abnormal reaction, but the enzymes of the intestine show a greater or less resistance towards differences in reaction, a remarkable adaptation to the conditions of their environment, because the reaction in the upper duodenum changes often and suddenly.[1]

Zymase, the metabolic enzyme of the yeast, acts only, or best, in the presence of phosphates; of sodium mono- or diphosphate. Perhaps this is an example of a true activator, a matter which will be dealt with later on, but more probably the power of the phosphoric acid to hinder marked changes of reaction is the cause of the favorable influence of the phosphates, and this is therefore a further exemplification of the importance of reaction upon ferments.

4. Chemical Properties. Enzymes combine both with their substrate and their dissociation products. The specificity of the enzymes mentioned before suggests to us, that the individual enzymes must have chemical relations with this substrate. We have no reason to think

[1] H. Friedenthal: Zeitschr. f. allgem. Physiologie, 1 (1901).—N. P. Schierbeck: Skandinav. Archiv f. Physiol., 3 (1891).

that all enzymes belong to the same class of chemical compounds. It has already been shown that the enzymes are accompanied as a rule by proteins and nucleic acids. The difficulties of separating enzymes and proteins have led physiologists for a long time to regard enzymes as protein-like bodies, though Brücke [1] and others [2] were successful years ago in freeing enzymes from all traces of proteins. Perhaps steapsin is a fat-like compound, and sucroclastic enzymes sugar-like compounds, and perhaps they belong to a wholly different class. We know but little to-day about the chemical characteristics of enzymes.

Enzymes are salted out by such neutral salts as are capable of readily salting out proteins, uric acid, and many dye-compounds, and particularly by ammonium sulphate. It seems that the individual enzymes are precipitated at different concentrations of ammonium sulphate—aldehydase and erepsin, when the solution contains sixty per cent, and trypsin and invertin at complete saturation. These differences can be used practically for purifying and separating enzymes, but they throw no light on their chemical nature. Enzymes are precipitated by alcohol, and the individual enzymes apparently by different concentrations of alcohol. Enzymes are precipitated, further, by heavy metals like uranium, and they are precipitated by tannin and by

[1] E. Brücke: Wiener Akad., Math.-Naturw. Klasse, 1861.

[2] J. Cohnheim: Virchow's Archiv, 28 (1863).—M. Jacoby: Zeitschr. f. physiol. Chemie, 30 (1900).

phosphotungstic acid. The enzymes can be dissolved again immediately after precipitation, but on standing they readily lose their solubility. Color tests for enzymes are not known.

It is of practical importance that enzymes are neither precipitated nor destroyed by many antiseptic substances, *i.e.*, by substances that kill and destroy living cells or check their growth. Chloroform, toluene, thymol, and ether, which dissolve the lipoid substances in cells and bacteria, and therefore destroy structures necessary to life, exert no influence on enzymes, because the enzymes are completely soluble in water, and do not yield any lipoids. It is very difficult to get extracts of living tissues, which cannot be heated or boiled, free from bacteria; so far as concerns the liver and pancreas, which yield bacteria in life, it is impossible.

During life, growth of micro-organisms is prevented or controlled in organs, but after death and loss of structure, bacteria grow in the fluids rich in proteins, carbohydrates, and suitable salts. These bacteria split the proteins, carbohydrates, and fats, as do the hydrolytic ferments, and give rise, like zymase, to carbon dioxide. Every one who has worked with ferments knows how easily we can be deceived when led to suppose ferments to be present when in fact we are dealing with bacteria. We distinguish between the two when we allow ferments to work for only two or three hours, because the action of ferments proceeds very quickly, whilst the growth of bacteria, even under the most favorable conditions, takes much more time.

But during extraction and purification, and if we extend experiments for a long time, it is absolutely necessary to protect enzyme solutions against bacteria by means of antiseptic substances. Amongst these chloroform or toluene have been most used within the last few years, and for the study of the enzymes of the alimentary canal crowded with bacteria, I suggest using both substances simultaneously. Thymol and ether have no efficiency. I think that all observations on enzymes, made without any addition of antiseptics, must be regarded with doubt and distrust. Other antiseptics, which coagulate proteins, such as fluorides, potassium meta-arsenite, and mercuric chloride, seem to have an injurious effect on the action of the enzymes. But no evidence has been brought forward showing whether ferments are precipitated and destroyed by these compounds, or whether the proteins, on coagulating, absorb and withdraw the enzymes. Buchner [1] has observed that zymase does not lose power in the presence of potassium meta-arsenite or ammonium fluoride in small amount, and that the proteolytic ferments of leucocytes are not harmed by corrosive sublimate. But these solutions, zymase and the extracts of leucocytes, are rich in protein, and the harmful influence of the mercury or the arsenite is checked by their union with protein. Purified enzyme solutions have not yet been studied with regard to their sensitiveness to these antiseptics, but for most practical purposes the lipoid antiseptics are thoroughly satisfactory.

[1] E. H. Buchner: "Zymasegärung," Munich, 1903.

CHAPTER IV

Enzymes as Catalyzers

We know of chemical processes in which the reacting substances completely disappear, giving rise to a new body. But in other processes one chemical compound reacts with another compound and changes it without entering into the final reaction itself. For instance, the conversion of cane sugar is expressed by the equation:

$$C_{12}H_{22}O_{11} + H_2O + n\text{HCl} = C_6H_{12}O_6 + C_6H_{12}O_6 + n\text{HCl}.$$

The hydrochloric acid causes the reaction, but before and after the reaction we find the same quantity of acid. A second case is the formation of ether from alcohol by sulphuric acid:

$$2C_2H_5OH + nSO_4H_2 = C_2H_5 . O . C_2H_5 + H_2O + nSO_4H_2.$$

The sulphuric acid is the cause of the reaction, but neither the original alcohol nor the end-products of the reaction contain the sulphuric acid, and the most important point is the fact that there is no quantitative relation between the hydrochloric acid and the sugar or between the sulphuric acid and the alcohol and ether. Chemists [1] apply the term "catalytic" to such reactions, and it seems that all enzymotic processes are catalytic reactions. The

[1] G. Bredig: Ergebnisse der Physiologie, 1, Biochemie (1902).

evidence for their designation as catalyzers is that we cannot find any relation between the quantity of enzyme and the quantity of matter decomposed by it, and that, after the reaction, we find the enzyme is not consumed. The last argument, however, is not completely proved, because the estimation of the enzymes is not so exact as to exclude the consumption of a portion.

The great discrepancy between the small quantity of enzymos and the enormous quantity of matter converted by enzymes, is astonishing, and has impressed all investigators. The best explanation seems to be the admission of an intercalated reaction assumed by chemists generally in the case of the formation of ether. Thus the reaction would be expressed by two equations:

1. $C_2H_5OH + SO_4H_2 = C_2H_5\,(HSO_4) + H_2O.$
2. $C_2H_5\,(HSO_4) + C_2H_5OH = C_2H_5.O.C_2H_5 + H_2SO_4.$

That is to say, the molecule of the sulphuric acid is combined first with one molecule of alcohol, then it combines with a second molecule of alcohol, and finally it is liberated and can attack a new molecule of alcohol. It is highly probable that enzymes are combined thus with the substrate they work upon and with the products they yield. The arguments for this supposition will be cited later on, but since I have spoken of the enzymes as catalyzers, the theory of Ostwald and Bredig should also be mentioned. They have ascribed a new function to catalyzers besides the quality mentioned. They define a catalyzer as a substance that never causes a reaction, but

only accelerates a reaction going on spontaneously but more slowly without the catalyzer. They propose the theory that the proteins or carbohydrates are always slowly dissociated in watery solution, and that the enzymes hasten this dissociation. They thought that through this theory they could avoid the difficulty presented by the fact that enzymes mediate in such extensive processes, extensive also as regards the energy involved. They argue that our body produces its whole energy through the enzymes oxidizing and burning the food, and that it is difficult to suppose that the great quantity of energy—four large calories per gramme of glucose, and nine per gramme of fat—can be liberated by the small quantity of enzymes which are available. The argument of Ostwald and Bredig has been adopted by many investigators; in fact we may say that it has ruled the whole theory of enzymotic action in recent years, but it seems to me that it does not hold good, and for three reasons:

1. It is not true that proteins or carbohydrates spontaneously undergo a slow dissociation in solution. Such solutions have been preserved for seventeen years or more at room temperature, and, in the absence of micro-organisms, of acid, or of alkali, the substances have been found unchanged. We have no reasons for thinking that the time of observation is too short, and that we would find a dissociation in centuries.

2. If we heat cane sugar with acid, it is decomposed in the same manner as by invertin, and we could here attribute the decomposition really to the H-ions, which

neutral water contains in lowest concentration, and assume simply that the enzyme hastens it. But in other cases, the enzymes cause other reactions than the H-ions of the water would bring about. The dissociation of glucose produced by H- or OH-ions gives rise to lactic acid and humins, while the dissociation by zymase produces alcohol.

3. Sugars and fats are oxidized and burned in the tissue, and set free much energy, but we do not know exactly to what extent this oxidation is caused by enzymes, and especially by one enzyme. It is possible that the structure of cells is needed for the complete combustion, and that two, three, or more enzymes work consecutively and perhaps in separate localities. We know that zymase is the only enzyme that gives off heat, and only a very small quantity of heat. The well-known enzymes, the hydrolytic ones, liberate no heat. The action of trypsin was measured by Grafe [1] in Rubner's laboratory with a calorimeter of high sensitiveness. He found no rise of temperature. The other enzymes have not been measured in this manner, so far as I know, but the heat of combustion of starch, the disaccharides, and of glucose, has been determined, and we can calculate that the differences lie within the errors of analysis. The building-up of protein from the amino-acids, or of the polysaccharides from the hexoses, as well as the accompanying dissociations, seems to be unconnected with any perceptible thermic process.

[1] E. Grafe: Archiv f. Hygiene, 62 (1907).

It is of great importance for the understanding of enzymes, that the hydrolytic enzymes neither give out nor consume any considerable or perceptible quantities of heat. Opinions regarding energy have here no weight.

We must see whether well-observed facts support the theory that enzymes are like inorganic catalyzers, and only hasten reactions without provoking them. For all proteolytic enzymes no fact is known which gives any evidence for it, and the same is true of ptyalin, invertin, lactase, zymase, and the corresponding metabolism enzymes urease and nuclease. On the other hand, evidence has been brought forward that esters like fats are dissociated spontaneously by the ions of the water, and that steapsins or lipases which split up fats and other esters, only hasten this process. Later on, in treating of the synthetical action of enzymes, I shall discuss the steapsins, or lipases, which are distinguished from all other enzymes, because they alone effect synthesis to any great extent. I think that the capacity for acting synthetically is connected with the property of the steapsins to hasten only a slow spontaneous action. The steapsin seems to have the properties of catalyzers in the sense of Ostwald and Bredig, and this property lies at the bottom of the synthetical action. According to the most important work of Pottevin,[1] the relations between dissociations and synthetical action in steapsin are perfectly similar to the relations between alcohols, acids, and esters in watery solution without

[1] Pottevin: Annales de l'Institut Pasteur, 20 (1906).

enzymes, because both reactions show an equilibrium-point, which is moved in the one or the other direction according to the temperature or the presence of more or less water. If ethyl alcohol is heated with acetic acid, the following reaction occurs:

$$CH_3COOH + C_2H_5OH = CH_3CO.O.C_2H_5 + H_2O.$$

But if water and ethyl acetate are mixed, the opposite reaction takes place, as follows:

$$CH_3CO.O.C_2H_5 + H_2O = CH_3COOH + C_2H_5OH.$$

If these two substances, alcohol and acetic acid, are allowed to mix, both reactions must proceed simultaneously in the same solution until they reach an equilibrium-point, that is to say, the point at which both proceed at the same rate, and all change seems to be stopped. The position of the equilibrium-point depends upon the mass of the three reacting substances, water, acid, and alcohol, and it depends, further, upon the temperature of the solution. The equation is therefore expressed in this manner:

$$CH_3COOH + C_2H_5OH \rightleftarrows CH_3CO.O.C_2H_5 + H_2O.$$

The steapsin moves the equilibrium-point, and thus acts like a true catalyzer. In concentrated solutions it hastens synthesis, but if we add water, it hastens dissociation. It is remarkable now, that the disaccharides, maltose and isomaltose, are glucosides according to E. Fischer, and are similar to ether in structure, and that he even has observed strong hydrochloric acid to have a synthetical influence on glucose. Compounds like isomal-

tose are thus formed. No evidence has been brought forward thus far regarding the slow dissociation of maltose in the absence of maltase, but perhaps we can expect this fact to be observed, and in that case maltase must be likened to steapsin and not to sucroclastic enzymes which attack the anhydrous polysaccharides like starch or cane sugar, and which exhibit no catalytic quality.

A second class of enzymes, which hasten only those reactions proceeding slowly without them, are the oxidases of the type of laccase. The more important metabolic enzymes, like zymase or lactacidase, I repeat emphatically, provoke conversions which do not occur at all in the absence of enzymes, and so do all proteolytic enzymes and the nucleases.

Bredig, Henri, and others have compared the time velocity of reactions, which are produced by inorganic catalyzers and by enzymes, and they have found that the curves resemble one another, but show some differences. I have already stated that it is impossible to study the laws of action, if we have only solutions so crowded with impurities as are our enzyme solutions. I have said, and may repeat again, that enzymes are modified in all sorts of ways in solutions, and shall presently mention that the action of enzymes is checked by the products formed by their action, and occurring therefore in the solutions; while in the case of ether or cane sugar, the acids provoking reactions are not influenced by substances appearing in the solution. Thus irregular curves result, which throw light upon the happenings in enzymotic processes.

We can only observe that the action of enzymes begins immediately when they touch their substrate, and that the process, with suitable reaction and temperature, proceeds most rapidly. If we add saliva, containing ptyalin, to starch, reducing sugars can be detected as soon as the fluids are mixed. Fibrin is dissolved in gastric juice, containing pepsin, as rapidly as sugar dissolves in water. If we allow active trypsin to act upon casein or other easily digestible protein, tyrosine is split off in so short a time that it resembles a mere precipitation or crystallization of the insoluble amino-acid. When studying arginase, Kossel wished to heat a liver extract with an enzyme in order to destroy arginase, and he set the tube in boiling water. The short time before the extract became heated was sufficient for the arginase to act upon the arginine and to split off some urea. The glycolytic enzyme of muscles does not convert more sugar in twelve hours than in two. The quick beginning and the rapid progress is a special feature of enzymotic action. If I find a ferment that acts only after a long time, proceeds slowly, and forms end-products only in small quantity, I always conclude that either the enzyme is of no great importance, or is not present under suitable conditions.

CHAPTER V

The Reversible Action of Enzymes

We know of many synthetic processes in the animal body which are opposed to the hydrolytic processes caused by enzymes. We can extract from the liver a diastase and a maltase dissociating glycogen into maltose, and maltose into glucose; the normal active liver can again convert glucose into glycogen. We can extract from the kidney the histozyme which splits hippuric acid into glycocoll and benzoic acid, while the living or surviving kidney builds up hippuric acid from the cleavage products.[1] The mucous membrane of the small intestine secretes a steapsin which decomposes fats; and the mucous membrane builds up neutral fat from the fatty acid and the glycerin entering the cells. Proteins are formed in the body from the amino-acids or from material still further removed from the protein. Plants decompose and build up cane sugar and starch according to their needs, and lecithin and nucleic acid arise in the developing egg from substances which are quite different.[2] For a long time it was thought that perhaps enzymes could also cause these opposite synthetic reactions. For the theory which

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[1] G. Bunge and O. Schmiedeberg: Archiv f. exper. Path. u. Pharm., 6 (1876).

[2] A. Kossel: Zeitschr. f. physiolog. Chemie, 10 (1886).

regards enzymes as catalyzers, and enzyme-reactions as equilibrium-reactions, it seems necessary to assume the synthetic action of enzymes.

The first to report an experimental proof of a reversible synthetical action of an enzyme was Croft Hill,[1] in 1898. He observed that in a concentrated solution of glucose (forty per cent), treated with a water-extract of yeast containing maltase, the rotatory and reducing power changes after a few weeks. The fact has been supported by Emmerling,[2] and it seems to be certain that extracts of yeast and plants containing maltase convert glucose in highly concentrated solutions to a greater or less amount, and give rise to a disaccharide and to dextrin-like bodies.

The nature of this synthetic disaccharide is debated; it seems that it is not the maltose hydrolyzed by maltase, but an *isomeric* substance, isomaltose, occurring also during the hydrolysis of starch and glycogen, but not hydrolyzed by maltase. We would have here, therefore, a synthetic action, but not a reversible action of the maltase. Strong hydrochloric acid seems to act in the same manner as the synthetizing enzyme. E. Fischer and Armstrong[3] made a similar observation on treating concentrated solutions of glucose with lactase, *i.e.*, with an extract of kefir grains. They found a disaccharide, not the lactose,

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[1] A. Croft Hill: Transactions of the Chem. Soc., 73 (1898).—Ber. d. deutsch. chem. Ges., 34 (1901).

[2] O. Emmerling: Ber. d. deutsch. chem. Ges., 34 (1901).

[3] E. Fischer and E. F. Armstrong: Ber. d. deutsch. chem. Ges., 35 (1902).

milk sugar, but an isomeric compound resembling it, which they named isolactose. This isolactose is not hydrolyzed by the lactase, which builds it up. According to Buchner,[1] a great amount of the glucose added to the press-juice of yeast is dissociated into alcohol and carbon dioxide, but another portion is converted into dextrin-like bodies. Cremer [2] observed that in the cell-free press-juice glycogen is formed from glucose.

We are much better informed regarding the synthetic action of the fat-splitting enzymes. Kastle and Loevenhart [3] were the first to observe that in a mixture of butyric acid and ethyl alcohol, ethyl butyrate is formed when a fresh pancreas extract containing steapsin is added. A control experiment carried out with boiled pancreas extract gave no ester whatever because the ethyl butyrate, like other ethers, is hydrolyzed by the steapsin of the pancreas. Here the synthesis and the hydrolysis involve the same compounds. The observation of Kastle and Loevenhart was confirmed by several observers, and in 1906 Pottevin,[4] in Paris, succeeded in obtaining a synthesis of the true fats in large amount by means of the steapsin or the lipase of pancreas. He treated finely divided pancreas with alcohol and ether, and thus prepared a fine dry powder which was insoluble in water, but which contained the lipase. Then he made a mixture of 100

[1] E. and H. Buchner and M. Hahn: "Die Zymasegärung," Munich, 1903.
[2] M. Cremer: Ber. d. deutsch. chem. Ges., 32 (1899).
[3] J. H. Kastle and A. Loevenhart: Amer. Chem. Journ., 24 (1900).
[4] Pottevin: Annal. de l'Institut Pasteur, 20 (1906).

gm. pure oleic acid, a little water, and the calculated quantity of glycerin, and added 2.5 gm. of the pancreas powder. The mixture was kept from three to twenty days at 33° C. In that time 85 gm. of the oleic acid were converted into mono-olein; in another series, more than 10 gm. of triolein were formed. Controls demonstrated that without the pancreas-powder, or with boiled pancreas-powder, only a small amount of the ester (four per cent as against eighty-eight per cent) was formed, and that the velocity of the reaction depends upon the quantity of the enzyme powder. If more water is added, the quantity of the ester formed diminishes, and in dilute solution the mono-olein and triolein are hydrolyzed by the same lipase. The reaction induced by the lipolytic enzyme is a strictly reversible one.

The proteins, peptones, and peptids are not built up by enzymes, nor, so far as we know, are urea, hippuric acid, or cane sugar. It seems that the reversible action occurs only with esters, that is to say, with compounds which, themselves, and without any enzyme, show a typical reversibility, a typical equilibrium-reaction. The equilibrium-point in dilute solutions approaches the limit of dissociation, while in concentrated solutions it approaches the other side of the equation. The enzymes accelerate the reaction or move the point of equilibrium farther in one direction than it would lie without them. The fats are esters, and it was shown by Nencki [1] that lipases or

[1] M. Nencki: Archiv f. exper. Pathol. u. Phar., 20 (1885).

steapsins also decompose many other esters. Kastle and Loevenhart, Pottevin, and others have observed that they also synthetize other esters from dissociation-products. The disaccharides, or maltose, isomaltose, lactose, and isolactose, are, according to E. Fischer, glucosides, or aldehyde-sugars, and their combinations are ethers, resembling the esters in behavior. Cane sugar and the higher polysaccharides are anhydrous sugars, and not ethers, and with them no synthesis has been observed.

We must very carefully separate enzymes into two classes. The one class, the catalytic, exhibits synthetic power, and it is possible that the animal body uses them for building up fats and other compounds. With the other class, not the slightest trace of synthesis has yet been observed.

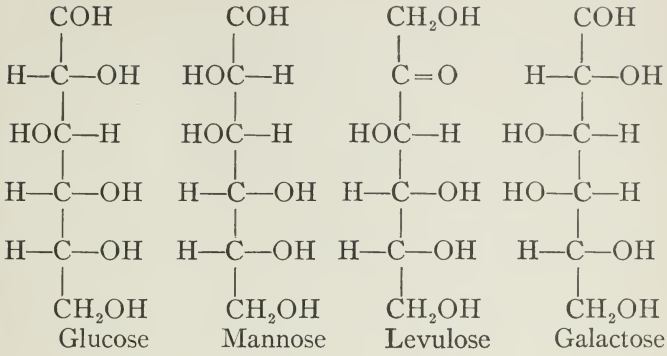
CHAPTER VI

Enzymes and Optical Activity

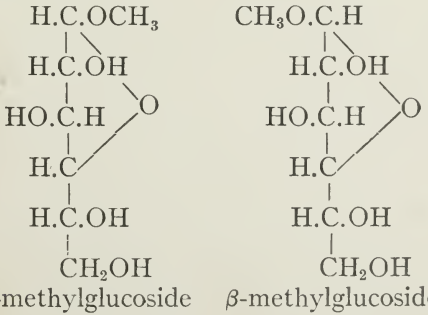
Pasteur was the first to observe that the two optically active forms of a chemical compound can differ in their behavior during metabolism. He noticed that micro-organisms, *e.g.*, *Penicillium glaucum*, attack only one of the two isomers of tartaric acid, and leave the other unattacked. Later, E. Fischer[1] discovered the constitution of sugars; he was able to build up synthetically all known hexoses, and many others not occurring naturally, and he threw light upon the constitution of the disaccharides. After this, he studied the action of the hydrolyzing enzymes of yeast and the higher animals, and the enzymes of yeast causing fermentation. The sixteen possible stereo-isomeric hexoses differ only by the position of the H and OH in space, and Fischer found that of the common hexoses only four are fermented by yeast. I here give the graphic formulæ of these four sugars (see facing page).

Glucose, mannose, and levulose do not differ in their four last carbon atoms. Galactose presents a slight difference in the fourth atom, and we find it is fermented

[1] E. Fischer: Ber. d. deutsch. chem. Ges., 23 (1890); 27 (1894). The enzymes are treated of in vol. 27 and 28, and Zeitschr. f. physiol. Chem., 26 (1898).



more slowly than the other three. All the other known possible hexoses are left unaffected by yeast. Still clearer are the relations between optical activity and the hydrolyzing enzymes. There are three enzymes which dissociate disaccharides—invertin, maltase, and lactase. According to E. Fischer, in the disaccharides hexoses are linked as in the glucosides, and he has built up from glucose and methyl alcohol two artificial stereo-isomeric glucosides resembling the natural glucosides, the α- and β-methylglucosides.



The two glucosides are identical except in the spatial or steric position of the radicals in the first carbon atom. Of these two compounds, invertin splits the α-glucoside and lactase and emulsin the β-glucoside. Both leave untouched the compound optically opposite. E. Fischer has also built up the two active ethyl-glucosides and they are decomposed, the α-glucoside by the one, and the β-compound by the other, enzyme. The glucosides occurring naturally in plants are β-glucosides; they are all decomposed only by emulsin or by lactase, and not by invertin.

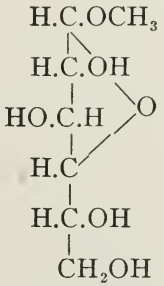
E. Fischer [1] has also built up, by linking amino-acids, chains which he calls "peptids." The amino-acids arising from proteins are optically active. For the synthesis Fischer took both the amino-acids occurring in nature, and the optically opposite forms, and also inactive racemic compounds. In studying the action of proteolytic enzymes, trypsin and erepsin, on these bodies, he found that they attacked only those compounds which correspond to the substances occurring in nature. The optically opposite form is not affected, and only half of the racemic form is decomposed. These relations are analogous to those pertaining to carbohydrates.

The large protein molecule exhibits two ways of linking amino-acids, the amino-linking which occurs in the peptids, and the amino-linking which unites urea and ornithine in arginine. The arginine has its own enzyme,

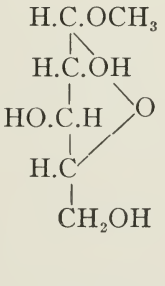
[1] E. Fischer: Ber. d. deutsch. chem. Ges., 33–42, and Fischer: "Untersuchungen über Aminosäuren, Polypeptide und Proteïne," Berlin, 1906.

arginase, discovered by Kossel and Dakin.[1] The natural arginine and one of its cleavage-products, ornithine, are dextro-rotatory. The arginase decomposes only this dextro-rotatory arginine. If we take racemic arginine, only half is split, and Kossel and Riesser[2] were thus able to isolate the levorotatory arginine until then unknown.

The efficiency of enzymes, at least of the enzymes that attack optically active substances, depends upon the steric configuration of the molecules they attack. But the steric configuration is not the only restriction by which they are bound. The α-methylglucoside, which I have mentioned, contains glucose, a hexose; and Fischer has built up in a similar manner the α-methylxyloside, in which methyl alcohol and xylose, a pentose, are linked together. I give here the formulæ of the glucoside and of the xyloside. We see that the xyloside differs only by the absence of the sixth, or perhaps better the fifth, C-atom.



Glucoside

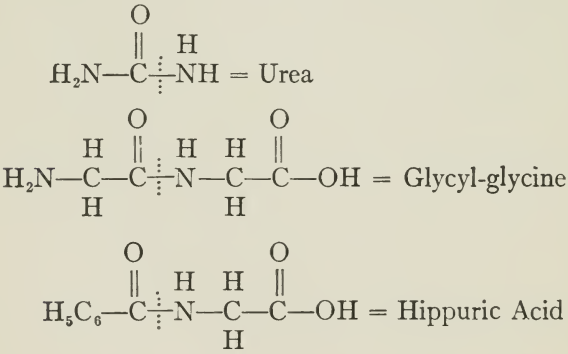


Xyloside

[1] A. Kossel and H. D. Dakin: Zeitschr. f. physiol. Chem., 41, 42 (1904).
[2] O. Riesser: *Ibid.*, 49 (1906).

Invertin loosens the link between the glucose and the methyl alcohol, that is to say, it attacks the molecules at a place where no difference exists between the glucoside and the xyloside, nevertheless the invertin does not disintegrate the xyloside.

Another instance: In the organism of the higher animals, we know of three combinations between the COOH-group and the NH_2-group, with loss of water. In the three cases the two groups are themselves linked together in the same manner, but the substitutions in these groups differ. Here are the formulæ for urea, glycyl-glycine, the simplest peptid, and hippuric acid.



The three compounds are split at the place I have indicated and we can see no difference in the molecules directly next the line of splitting. In the case of urea, we have merely the NH_2-group; in the other compounds one H is substituted, the right side being the same in the glycyl-glycine and the hippuric acid. Nevertheless, every one of the

three compounds has its own individual enzyme. The urea is decomposed by certain bacteria containing urease. The hippuric acid is dissociated by histozyme, an enzyme found in the kidney by Schmiedeberg.[1] The glycyl-glycine is attacked by erepsin, an enzyme produced by the small intestine and other tissues. The histozyme cannot affect the peptids, nor can the erepsin attack hippuric acid.[2] The urea is built up in the liver, the hippuric acid in the kidney, and the peptids in the growing tissues.

The enzymes and the substrate they work upon are in close chemical relationship. E. Fischer has illustrated it by saying that enzyme and sugars are mutually related to one another in the same way as a key to the lock which it alone can open. Both the chemical configuration and the steric, the atomic relation in space, form the lock. Compounds resembling one another in both features are opened by the same key. Erepsin, and in a lower degree trypsin, dissociate all peptids, because in them all the amino-acids are linked in the same way as in the glycyl-glycine. We may regard all peptids as derived from glycyl-glycine by substitution of an H by a radical like C_4H_9 (leucine), or C_7H_8 (tyrosine), etc. The dissociation always takes place at the already existing *locus minoris resistentiæ*, common to all peptids, peptones, and albumins. In the case of the peptids and proteins, the action or non-action of enzymes can be deduced from the known chemical

[1] O. Schmiedeberg: Archiv f. exper. Path. u. Phar., 14 (1851).

[2] E. Abderhalden and Y. Teruuchi: Zeitschr. f. physiol. Chem., 49 (1906); O. Cohnheim: *ibid.*, 52 (1907).

structure, and we understand also why the sucroclastic enzyme of yeast, the zymase, ferments only the four sugars resembling one another and not the other artificial hexoses with greater differences in the other C-atoms. But we do not understand why the same enzyme, invertin, which I have said dissociates α-methylglucoside, splits up also cane sugar, and why lactase decomposes both the glucosides and a compound so widely different as milk sugar. The third enzyme affecting disaccharides, *e.g.*, maltase, attacks only maltose, and none of the glucosides synthetically obtained. It suggests to us that our chemical formulæ written only on the plane of the paper, do not represent all the space relations of the molecular structure.

We can make observations on the fate of some aromatic amino-acids in metabolism. We shall see later that with great probability all dissociations in the body can be ascribed to enzymes, that metabolic enzymes oxidize food, and change food without oxidation. If this suggestion is right, we can draw conclusions from what takes place in metabolism regarding the qualities of the enzymes causing the changes. We observe that of all existing amino-acids, the α-amino-acids alone disappear completely in the human or animal body, if given by mouth or subcutaneously. Tyrosine or oxyphenylalanine and phenylalanine are substituted alanines, and α-alanines. Tyrosine, phenylalanine, and all investigated compounds which are substituted at the α-C-atom, phenyllactic acid, or compounds with a keto group at this atom, are oxidized

completely. On the contrary, compounds which are formed by substitution at the β-atom, are not attacked, or they are changed slowly, in small amount and in successive stages. Some investigators, like Knoop,[1] have thought them suitable for studying the course of metabolism in the body; but I think we must be very cautious in drawing conclusions from these abnormal compounds. If we cast a glance at the formulas generally adopted, we must suppose that α- and β-phenylalanine closely resemble each other, and further, that the differences must be greater between α- and β-isocapronic acids than between the α-aminoisocapronic acid and aminolactic acid. Nevertheless the same enzyme attacks both the amino-acids and leaves untouched the β-acids. We must suppose that the same benzene-nucleus, C_6H_6, is yielded by phenol, benzene, or phenylalanine. And if we use only inorganic chemical means, we find no great difference, but if we use enzymes as reagents, we see that benzene and phenol pass unchanged through the body and that only in phenylalanine is the aromatic cycle broken. The enzymes exhibit to us differences not only in the side-chain, but in the cycle itself. We shall see later that we must probably distinguish between the combination and oxidation in the body, which sets free energy, and the slow conversion without combustion, which is used for special functions. The enzymes which produce the first effects, attack at once the whole molecule, and they enable

[1] F. Knoop: Hofmeister Beitr., 6 (1905).

us thus to understand the intimate structure of the molecule more thoroughly.

Not all enzymes are so specific as the proteolytic or the sucroclastic enzymes already mentioned. It seems that proteins and poly- and disaccharides are never burned by animals, plants, or bacteria, without being decomposed into monosaccharides and amino-acids. Cane sugar and milk sugar entering our body without intervention of the alimentary canal, are eliminated quantitatively. Proteins which are not previously dissociated are protected against bacteria. Yeast fed on pentoses, or on hexoses other than the four I have mentioned, starves as if not fed at all. But the tissue enzymes mediating the metabolism of the higher animals do not show so great a difference. If we feed a man or a dog with pentoses or strange hexoses, he eliminates them in the urine, but not all that was absorbed. The rest disappears; it must be burned in metabolism. Amino-acids and peptids optically opposite to the natural, are eliminated in part; another part is utilized in the body.[1]

The natural compounds are preferred by the metabolic enzymes, and are completely burned up, but these enzymes can also attack other substances. The optical antipodes of peptids, given in small quantity, can even disappear completely in the organism of the dog or rabbit. Thus we see that men burn alkaloids, medicaments, and other artificial substances for which they never can produce

[1] E. Abderhalden, P. Bloch, and P. Rona: Zeitschr. f. physiol. Chem., 52 (1907).

enzymes. The enzymes of metabolism have not a complete specificity, but a relative one. These keys open locks only more or less resembling the locks for which they are destined. And if we follow the series of living things to the opposite end, to the simplest organism, we find some bacteria which live on almost any organic material. The natural sugars and the natural amino-acids are preferred even by them, but they are not necessary. Pfeffer [1] states that they are extraordinarily comprehensive in their activities, that is to say, their enzymes must open almost every kind of lock.

It will be seen later on, that these last enzymes have not been thus far isolated. The enzymes which are well known are specific, and their relation to the optically active compounds throws light on the chemistry of the enzymes themselves. The enzymes must be optically active compounds. E. Fischer has discovered that the two stereoisomeric components of a substance show differences only in their relations to optically active compounds, and not to other substances. It often happens that the inactive or racemic form has quite other properties than those of the two active forms. There are deviations in melting-point, solubility, taste, and other properties, but the two active components resemble each other in all ways, except in their rotatory power and in their combinations with other active bodies. For instance, the two optically active compounds of benzoyl-aspartic

[1] W. Pfeffer: "Pflanzenphysiologie," vol. 1, p. 349.

acid and their salts with sodium or potassium are alike except in rotatory power, but the salts of this acid combined with optically active bases, such as the components of brucine, strychnine, or quinine, show such differences in solubility as to enable us to separate the two salts from each other. It seems to be a general rule, that only combinations of two optically active compounds differ in this way, and we must conclude, therefore, that the proteolytic and sucroclastic enzymes contain an unsymmetrical carbon-atom.

CHAPTER VII

Mode of Action of Enzymes

The close relationship between enzymes and their substrate mentioned in the last chapter, brings evidence that the enzymes enter into a chemical combination with the molecules they work upon. Another proof of this is afforded by the power of the substrate to protect enzymes. You have heard how easily the enzymes undergo destruction or loss of power by heat, by chemical processes, or spontaneously. Kühne and Biernacki [1] observed that proteins accompanying trypsin in solution increase its stability. In a purified solution of trypsin poor in protein, the enzyme loses its power at 40° to 45° C. in one hour, while the natural pancreatic juice or a fresh extract of the gland rich in nucleoprotein can be heated for one hour at 55° C. without damage, and is destroyed only at 60° to 65° C. The purer the enzyme the sooner does it lose its activity. Extracts of leucocytes contain a very small quantity of trypsin or of a proteolytic enzyme resembling trypsin, and besides that they contain protein in much greater amount. An extract of leucocytes can be heated to 56° C., and the enzyme resists formaldehyde, mercuric chloride, or normal sodium hydroxide.[2]

[1] E. Biernacki: Zeitschr. f. Biol., 28 (1891).—K. Mays: Zeitschr. f. physiol. Chemie, 38 (1903).

[2] G. Jochmann and G. Lockemann: Hofmeister's Beiträge, 11 (1908).

Pepsin [1] undergoes rapid destruction when separated from the nucleoprotein present in natural gastric juice. The protective power of the proteins can be explained in a more specific way; proteins neutralize acids, alkalies, formaldehyde, mercuric chloride, and other substances that might precipitate and damage the enzyme. But the increased resistance of the proteolytic enzymes to heat in the presence of proteins cannot be satisfactorily explained in this way.

Sucroclastic enzymes are likewise protected by sugars. O'Sullivan and Thompson [2] found that without cane sugar, invertin is almost wholly destroyed by heating to 50° C., and to a great extent by heating to 45° C. When cane sugar is present, it can be heated to 60° C. without losing any activity at all, and on heating to 70° C., it loses only a part of its power. If we add sugar to a solution of zymase, the latter is capable of causing the production of carbon dioxide

At 6° C., during 10 days
" 22° C., " 4 "
" 35° C., " 1 day

But if we try to preserve the press-juice of yeast without sugar, we find that the contained zymase loses its activity

At 0° C., in 2 days.
" 20° C., " 1 day.

[1] J. P. Pawlow: Nagel's "Handbuch," vol. 2 (1906); "Arbeit der Verdauungsdrüsen," 1898.

[2] O'Sullivan and Thompson: Journ. Chem. Soc., 1890.

For other enzymes no evidence on this point has been secured, but from the fact that in natural pancreatic juice steapsin and nuclease undergo deterioration in a very short time, while in the intestine the digestion of fat and nucleic acid continues for many hours, we cannot doubt that the law of protection by the substrate holds good for them also. The enzyme is changed by the substrate it works on, and the solution does not contain the same enzymic compound as the pure solution, but it contains another chemical compound of different properties, and this compound must be an enzyme-protein, an enzyme-sugar, etc. It is a chemical combination of enzyme and substrate. The higher resistance is an evidence of the existence of such combinations.

Further evidence of the combination of enzyme and substrate is seen in the antitryptic action of serum albumin found by Hahn [1] and explained by Hedin.[2] A tryptic solution digests fibrin, but it cannot do so if serum or serum albumin is added. The trypsin is then bound by the serum albumin. The serum albumin, which is dissolved with difficulty by trypsin, takes away the enzyme from the easily digestible fibrin, and thus the digestion of fibrin is checked, *i.e.*, the enzyme is side-tracked.

In other cases, the true chemical combination of enzymes, particularly of the proteolytic enzymes, with the substrates is not so clear, because the chemical combination is complicated by the physical adsorption above mentioned.

[1] M. Hahn: Berlin. klin. Wochenschr., 1897.

[2] S. G. Hedin: Zeitschr. f. physiol. Chem., 52 (1907).

If we put fibrin in a solution of trypsin at zero, and observe that the fibrin takes up all of the trypsin, we must suppose —for the reasons above stated—a loose combination between the protein and the trypsin. But fibrin takes up sucroclastic enzymes, hormons,[1] etc., and trypsin is completely taken up by animal charcoal,[2] and to a certain extent by siliceous earth.[2] The relations are, therefore, not entirely clear.

Likewise, enzymes enter into combination with dissociation-products. It is an old observation that the substances produced by the action of an enzyme exert a retardation of that action. Experimentally, the fact is of the highest importance, because such retardation by its own activity is the reason that the digestion in the alimentary canal takes place under conditions very different from those which we obtain during artificial digestion in flasks and beakers. Trypsin and erepsin do not normally dissociate the protein in a flask, but do in the intestine; the resulting amino-acids are immediately absorbed, and the enzymes not passing the intestinal wall can freely attack new molecules of protein. In the tissues, the structure of the cells and the circulation of the blood favor the action of enzymes in a similar fashion. The diastase splits the glycogen and, immediately thereafter, the diastase and the sugar formed by it are separated from each other. Under these conditions a comparatively small quantity of enzymotic

[1] O. Cohnheim: Arch. des Sciences biolog. de St. Pétersbourg (11 Suppl.), (1904).

[2] S. G. Hedin: Zeitschr. f. physiol. Chem., 52 (1907).

fluid is able to dissolve a relatively large amount of the substance it splits. Lea[1] has compared the digestion of fibrin by trypsin in a flask and in a dialyzer tube under otherwise the same conditions and found, after six hours, a residue in the dialyzer tube of 1 gm., in the flask 2.5 gm.

In another experiment he mixed saliva and 10 gm. of dextrin, and afterward he found 0.4 gm. recoverable dextrin in the dialyzer tube, and in the flask 2.5 gm.

He unfortunately used weak solutions of trypsin. With stronger solutions the results would be still more striking. *In vitro*, peptic digestion produces chiefly proteoses, and peptones in small amount. In the living stomach, Tobler[2] found, under really normal conditions, twenty per cent proteoses and eighty per cent peptones. I have studied peptic digestion in dialyzer tubes, and was able, in nineteen hours, almost completely to dissociate 90 grammes of meat-protein into peptone. In such experiments, we are therefore able to avoid a good deal of the retardation above referred to.

But what is the reason of the retardation? In the case of those enzymes that act as catalyzers, which accelerate only one of the two reactions occurring without them, and which move the equilibrium point, the retardation can be explained easily. The accumulation of the products of dissociation increases one side of the equation, and when the equilibrium point is approached, the reaction

[1] A. S. Lea: Journal of Physiology, 11 (1890).
[2] L. Tobler: Zeitschr. f. physiol. Chemie, 45 (1905).

proceeds more and more slowly. But as I have already stated, the theory of the catalytic nature of enzymes holds good only for fats and fat-splitting enzymes, and the explanation just stated does not apply to the retardation observed with proteolytic and sucroclastic enzymes, because the enzymotic reactions are retarded and checked not only by their own dissociation products, but by other products resembling the substrate and dissociation-products in chemical and steric configuration. The ptyalin of the saliva decomposes starch and produces maltose, but the retardation is exerted not only by maltose, but also by glucose,[1] which does not appear in the equation expressing the reaction. Further evidence for this has been advanced by Abderhalden and Gigon.[2] They allowed the proteolytic enzyme of yeast to work on the optically active peptid, glycyltyrosine. The enzyme splits the glycyltyrosine into glycocoll and tyrosine, according to the equation:

$$H_2NCH_2CONHCH(C_7H_6OH)COOH + H_2O = H_2NCH_2COOH + H_2NCH(C_7H_6OH)COOH.$$

The equation contains only glycocoll and tyrosine, and if the law of equilibrium holds for enzymes, we must expect that only glycocoll and tyrosine would retard the reaction. But Abderhalden and Gigon have found that glycocoll, which contains no symmetrical carbon atom, exerts no retardation, but all other active amino-acids,

[1] J. Cohnheim: Virchow's Archiv, 28 (1863).

[2] E. Abderhalden and A. Gigon: Zeitschr. f. physiol. Chem., 53 (1907).

serine, alanine, leucine, tryptophane, etc., do. Thus the accumulation of end-products cannot explain the retardation, because the other amino-acids are not end-products of the reaction.

The only explanation I can give is that the enzymes combine with their dissociation-products just as they do with their substrates. As active compounds, they react only with active compounds, but they join with all compounds of the corresponding steric and chemical configuration. A further proof of this is that trypsin is protected against heat by peptones and amino-acids in the same way as by proteins.[1]

I suggest, for instance, that invertin is united with cane sugar in a loose chemical combination. The molecule of enzyme-cane-sugar and the enzyme can undergo combination with a new molecule of cane sugar, but it can also be abstracted by one of the newly-formed sugars, glucose, or levulose. The retardation is not governed by the law of equilibrium, but by the law of division. The enzyme can be side-tracked in the same way by either the substrate or the dissociation products.

If we picture to ourselves a chemical image of the mode of action of enzymes, we may consider that invertin enters the molecule of cane sugar, and that this new molecule becomes unstable and separates into two smaller molecules resembling the greater one, and also able to combine with the enzyme. This would be a strictly chemical explana-

[1] E. Biernacki: Zeitschr. f. Biol., 28 (1891).

tion of what occurs during enzymotic action, and we know of other analogous chemical reactions as, for instance, those in which a chemical compound of great stability is converted into a more unstable one by introducing an amino-group into the molecule.

But, as a matter of fact, the relations are probably more complicated, because the ferments are colloids, that is to say, we are not dealing with reactions occurring in a homogeneous medium, but with substances not really dissolved, but acting upon dissolved compounds. Meltzer[1] refers to the *organization* of ferments, which implies not a simple chemical arrangement inside of the molecule, but a complex which resembles the structure of living protoplasm, and consists of a close connection of fluids and solids forming a physical rather than a chemical unit.

[1] S. J. Meltzer: Amer. Journ. of Physiology, 1909.

CHAPTER VIII

Antiferments

Some authors [1] have described compounds which prevent enzymotic action in a specific way, just as antitoxins bind and check toxins. They have met with great difficulties in attempting to give convincing proofs for this suggestion. The action of pepsin is disturbed by neutralizing the acid or by preventing the swelling of the fibrin. The action of trypsin, diastase, and steapsin is retarded by the accumulation of the dissociation products, and the action of all enzymes, by the addition of the substrate, or adsorption by coagulating proteins, or spontaneous loss of power. Every substance or every condition which increases the time of conversion, or has a harmful influence on the enzyme or on the conditions of enzymotic action, appears to us as an antiferment. Some authors [2] have injected solutions of enzyme into the veins of the rabbit, and have observed that the blood of the rabbit then yields a precipitin, that is to say, a substance that gives a precipitate when added to the injected solution. In some cases enzymotic action in the solution is prolonged or diminished under the circumstances. But the ex-

[1] E. Weinland: Zeitschr. f. Biol., 44 (1902).

[2] J. Morgenroth: Zentralblatt f. Bakteriol., 1899.—H. Sachs, Fortschr. d. Medizin, 20 (1902).

periments are not conclusive because the solutions always contained proteins, and these alone would give rise to the formation of a precipitin, and the precipitate formed by it in the solution might withdraw the enzyme by adsorption. Hahn and Hedin (see above) have observed that the blood serum of mammals, and to a less degree the white of egg, retards or prevents the action of trypsin, and some authors have spoken of the antiferment present in the serum. I have already given a clear explanation of the phenomenon described by Hedin; the serum albumin as a slowly digestible protein appropriates the trypsin, and diverts it from the easily digestible fibrin, and thus protects the latter from action. Weinland observed that the mucous membrane of the stomach and the small intestine, and the round worm frequently present in the intestine, yield substances that retard or prevent the action of pepsin or trypsin. He suggested that these results were due to antiferments which have a great biological importance, because they protect the worms and the digestive organs against digestion. In the case of antipepsin there can be little doubt that Weinland's antipepsin is a protein or a salt which neutralizes the hydrochloric acid, and, also, in part, a neutral salt, which prevents the swelling of fibrin in the hydrochloric acid. In the case of the intestine and the worms, Weinland's view is not disproved, but there are some strong objections to it. Both the mucous membrane and the body of the worms contain proteins less digestible than the fibrin, which must divert the trypsin from the fibrin. It is remarkable that, according

to Hamburger and Hekma,[1] the intestinal juice can likewise disturb the digestion of fibrin, although it is, of course, absurd to imagine the secretion of an antitrypsin in the intestine.

Extracts of the small intestine also yield enterokinase, the activator of the trypsin. When we add increasing quantities of intestinal extract to a given quantity of pancreatic juice the digestive power is not proportionately increased. The first small quantities accelerate, the later additions retard, the action of the trypsin.

It seems to me that the evidence does not permit us to speak of specific antiferments.

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[1] J. H. Hamburger and E. Hekma: Journ. de Physiologie et de Pathologie générale, 1902.

CHAPTER IX

Specificity of Enzymes

It was a question debated for a long time whether the individual enzymes occurring in different animals and in plants are identical or not. The proteolytic enzymes ought to be different in different animals; for instance, fishes ought to have another pepsin than mammals, the trypsin of the dog and the ox should be different, etc. But we must make the same objections as in the case of the antiferments, that the small differences in the action of enzymes can be explained completely by the slight differences in the environment, in the method of obtaining of the enzymes, etc. Until we can isolate the enzymes as chemically pure compounds, we have no means of answering these questions with certainty, but in all probability the enzymes having similar actions are the same. The cells of our salivary glands, the protoplasm of yeast, and the germinated seeds of plants produce for the same purpose, *i.e.*, the digestion of starch, the same compounds: diastase and maltase.

CHAPTER X

Zymogens and Activators

Some enzymes are produced by the cells in such form that they do not need further supplement to render them active. This is the case with all enzymes which dissociate poly- and disaccharides, such as diastase, maltase, invertin, and lactase. An exception seems to be the glycolytic enzyme of the muscles, which appears to require an activator derived from the pancreas. Other enzymes independent of such aids are erepsin and the nucleases. The remaining enzymes are formed in the cells, and are secreted from the cells in an inactive, incomplete form. They meet with other chemical substances and combine with them to form the complete enzyme which is then capable of dissociating the appropriate substrate. We know the following examples of such enzymes:

1. The pepsin of the stomach is produced and secreted by the chief cells of the gastric glands as a zymogen, the pepsinogen. Grützner [1] found, in 1874, that extracts of the mucous membrane of the stomach, in activity or at rest, contain a substance which is not pepsin, but yields pepsin. It therefore received the name pepsinogen. Pepsin is destroyed by alkalies, and, according to

[1] P. Grützner and M. Ebstein: Pflüger's Arch., 8 (1874).

Langley, also by carbon dioxide; but pepsinogen is not. Because the same glands contain parietal or ovoid cells secreting the hydrochloric acid, the secreted gastric juice always contains pepsin. The acid changes the pepsinogen and is also necessary for the action of the pepsin formed from it. We do not know how the enzyme and acid are associated. The conversion of pepsinogen into pepsin is apparently not reversible.

2. Trypsinogen and Enterokinase. As Heidenhain [1] found in 1875, the extracts of the pancreas of the dog and the pig contain trypsin in small amount; the quantity increases if we first treat the extracts with acetic acid and then establish the correct alkaline reaction. Kühne [2] observed that even the dried pancreas powder yields trypsinogen, and Pawlow [3] showed that the secreted pancreatic juice often contains no trypsin, but trypsinogen, and that this trypsinogen is converted into trypsin by enterokinase, a compound produced by the mucous membrane of the duodenum and other parts of the small intestine, and present in the enteric juice. It is possible that trypsinogen and enterokinase combine with each other and thus form a new compound, the real trypsin. But it is also possible that the enterokinase converts trypsinogen into trypsin. Both views are supported by ex-

[1] R. Heidenhain: Pflüger's Arch., 10 (1875).

[2] W. Kühne: "Untersuch. a. d. physiol. Institut Heidelberg," 1 (1878).

[3] J. P. Pawlow: Nagel's "Handbuch," 2 (1906); Dissertat. of Schepowalnikow (1898); Lintwarew (1901); Sawitsch, Russki Wratch, 1 (1902).

perimental facts, but the facts do not enable us to give a clear decision. Bayliss [1] has added enterokinase to trypsinogen, and thus converted it into trypsin. When he added some of this solution to another solution of trypsinogen, the new trypsinogen was converted also. Bayliss concluded that enterokinase is not consumed in activating trypsinogen. It acts therefore like a ferment, and he suggests that enterokinase does not itself enter into the composition of the trypsin. Observations have been made, however, which support the theory of the linking together of the two compounds to form trypsin. When we add to a solution of trypsinogen increasing quantities of enterokinase, the tryptic activity of the mixture first increases, and then decreases, so that both substances must stand in a certain proportion to each other for the optimal efficiency, and it seems probable that they are combined. The actual process is not yet clear, nor is it known whether activation of trypsinogen by enterokinase and the activation by acetic acid, as observed by Heidenhain and Kühne, are the same or different processes, so that much has yet to be learned regarding this matter.

3. Lipase or Steapsin of the Pancreas and Liver and Bile Salts. Nencki,[2] Zuntz,[3] and Pawlow [4] observed that

[1] W. M. Bayliss: Arch. des Sciences Biolog. de St. Pétersbourg, 11 (1904).

[2] M. Nencki: Arch. f. exper. Path. u. Pharm., 20 (1886).

[3] N. Zuntz (and Ussow): Arch. f. (Anat. u.) Physiol., 1900.

[4] Lintwarew: Diss. St. Pétersbourg, 1901.—J. P. Pawlow: Nagel's "Handbuch," vol. ii. (1906).

the lipolytic action of pancreatic juice increases very much on adding bile, though the bile itself has no lipolytic action. This case of activation has been explained by the investigations of Magnus.[1] Magnus pointed out that the bile salts, sodium glycocholate and sodium taurocholate, in the bile, are the active compounds, and since the salts prepared by synthesis act in the same way, we are sure that we are not deceived by an impurity adsorbed by the natural bile salts. Magnus[2] further showed that the combination of the lipase—he used the more stable lipase of the liver, probably identical with the lipase of pancreas—with the bile salts can be separated by dialysis. The bile salts pass through the parchment membrane, while the lipase remains within the dialyzer tube, and thus loses its activity. If we add the bile salts, the activity is again restored, and the experiment can be repeated several times. Lipase and bile salts join together to form a loose compound.

4. Glycolytic Enzyme of Muscles and a Hormon of the Pancreas. If we add glucose to a fresh water-extract of cat-muscles, and allow the extract to stand a few hours at 38° C., we find the quantities of reducing sugars unchanged. But if we add extract of pancreas treated in a certain manner, we find after three or four hours less glucose than before. The detailed action of the glycolysis is not clearly understood.

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[1] R. Magnus: Zeitschr. f. physiol. Chem., 48 (1906).—A. S. Loevenhart: Journ. of Biol. Chem., 2 (1907).

[2] R. Magnus: *ibid.*, 42 (1904).

5. Lipase of Ricinus-Seeds and Acid. The germinated seeds of Ricinus, the castor-oil plant, contains a lipase which hydrolyzes fats and other esters, slowly in neutral solutions, but very rapidly on adding acid. During a certain period of germination, an organic acid is formed, and provokes the action of the lipase.[1]

6. Laccase and Manganese. According to Bertrand,[2] the ash of laccase, the oxidizing enzyme of the lac tree of East Asia, always contains manganese; the activity of laccase is associated with the presence of manganese, and the activity of an enzyme preparation is proportional to the manganese present.

7. A last case, perhaps, is the association of zymase and phosphates,[3] but as I have said before, we are here probably dealing with a mere improvement of reaction.

The advantage of this need of activation to produce the completed enzyme seems clear in the case of pepsin and trypsin, which, as zymogens, cannot act within the cells producing them. If they could act there, they would destroy the protoplasm, and if trypsin could work in the pancreatic juice, it would hasten the destruction of other enzymes not protected in the juice by their substrate. The lipase of the pancreas does not work without bile, and we know that bile dissolves fatty acids and enables

[1] W. Connstein, E. Hoyer, and E. Wartenberg: Ber. d. deutsch. chem. Ges., 35 (1902).—W. Connstein: Ergebnisse der Physiologie, iii., Biochemie, 1904.

[2] G. Bertrand, Compt. rend., 124 (1897).

[3] E. Buchner: Zeitschr. f. physiol. Chem., 46 (1905); Biochem. Zeitschr., 8 (1908).

them to be absorbed. Without bile, the fatty acids not soluble in water and not absorbed, would accumulate, and this concentration of fats would then check the hydrolysis or the process might even be reversed, synthesis of fats resulting.

In muscle the glycolytic enzyme oxidizes the sugar within the fibres, and it is there that the energy produced by this oxidation is utilized. But since the contents of the muscle fibre are fluid, it is difficult to separate sugar and enzyme, and in this case the requirements are met by the activation of the enzyme by acid as occasion may demand.

CHAPTER XI

The Individual Enzymes

The Hydrolytic Enzymes of the Alimentary Canal

This group is the best known of all, because the enzymes are secreted. We can thus study the natural juices, or easily extract the enzymes.

In man and the higher animals, the following fourteen or eighteen enzymes are known:

Diastase of the saliva.
Diastase of the pancreas.
Maltase of the small intestine.
Lactase of the small intestine.
Invertin of the small intestine.
Lipase of the stomach.
Lipase of the pancreas.
Lipase of the small intestine.
Lecithase of the pancreas. } Perhaps identical with
Lecithase of the small intestine. } the lipases.
Pepsin of the stomach.
Protease, peptone-splitting, of the pyloric part of the stomach.
Trypsin of the pancreas.
Erepsin of the small intestine.
Arginase of the small intestine. } Perhaps not digestive
Arginase of the liver. } enzymes.

Nuclease of the pancreas.

Nuclease of the small intestine.

The large intestine produces no enzymes.

The object of digestion is to bring food-stuffs into solution and to convert them into compounds which can pass through the intestinal wall. All carbohydrates are converted into monosaccharides, glucose, levulose, and galactose; all proteins into amino-acids; all fats into fatty acids and glycerin; lecithin into fatty acids, choline, glycerin, and phosphoric acid; and nucleic acid into pyrimidines, purines, phosphoric acid, and carbohydrate.

For securing this complete disintegration, each class of enzyme action is repeated two or three times while the food passes along the alimentary tract. If one enzyme fails, the disintegration can be effected by the following one. We have two diastases in the saliva and the pancreatic juice; three lipases; two enzymes, pepsin and trypsin, dissolving proteins, and three enzymes dissociating them: trypsin and both erepsins. Only the enzymes of the small intestine are simple, *i.e.*, require no activator, and I have spoken of their exceptional behavior, because they seem to act to some extent within the cells, and attack the compounds which pass through them.

Carbohydrate-Splitting Enzymes

The saliva, the first digestive fluid, contains diastase or ptyalin which converts starch and glycogen into maltose. Starch and glycogen are anhydrides of the sugars, and do not give the reduction reactions of sugars—the tests of

Trommer, Fehling, Pavy, or Almén-Nylander. With iodine, starch gives a blue, and glycogen a red reaction. The conversion can be followed by the disappeance of these color reactions, or by the appearance and increase of the reducing power. The transition is a very gradual one, owing to the existence of a number of intermediate substances, the dextrins and isomaltose. The isomaltose alone is a distinct chemical individual. The dextrins are mixtures which have not been separated into their constituents. Among them we can distinguish two groups by means of the color reactions with iodine: the erythro- and the achroo-dextrins. In the beginning of the conversion, starch loses its colloidal character, and, without changing its chemical properties, becomes readily soluble in water. The different reactions with iodine are as follows:

Starch, blue, no reduction.
Soluble starch, blue, no reduction.
Erythrodextrin, red, reduction.
Achroodextrin, no color, reduction.
Isomaltose, no color, reduction.
Maltose, no color, reduction.

The compounds or group of compounds appear and disappear one after another, but from the beginning of the conversion all dissociation products are present simultaneously. The real chemical nature of this process is not yet known. The conversion by ptyalin is the same as the conversion by dilute boiling acids. The only difference seems to be, that the enzymotic dissociation by

ptyalin stops at maltose, while starch boiled with acids is converted finally into glucose. The conversion of maltose into glucose is effected by a special enzyme, maltase, as Musculus and von Mering [1] pointed out in 1878. These enzymes, so closely related in their action, may be produced in the same cells; such is the case in the tissues, blood corpuscles, and in plants; but they are separated in the alimentary canal. Saliva and pancreatic juice contain no maltase, and the enteric juice contains maltase but no ptyalin, or only traces. The ptyalin of the saliva is, so far as we know, identical with the ptyalins of the liver, of the pancreas, muscles, malt, yeast, and other plants.

The mixed human saliva always contains a great deal of ptyalin, and so does the extract of the human submaxillary gland. The parotid saliva seems to be less active. In the pig and guinea-pig also all the salivary glands produce a large amount of enzyme; while in the rabbit only the parotid saliva is rich, the submaxillary saliva poor in enzyme. In other animals, ptyalin rarely occurs in saliva. Neither the saliva of the horse nor the extracts of the salivary glands of the large ruminant animals, the ox and sheep, appear to contain ptyalin. The saliva of dogs and cats shows at most an extremely feeble amylolytic power, often none at all; it contains much less enzyme than do the blood and lymph of the same animals.[2] Experi-

[1] F. Musculus and J. von Mering· Zeitschr. f. physiol. Chem., 2 (1878).—C. Hamburger: Pflüger's Arch., 60 (1895).

[2] L. B. Mendel and Frank P. Underhill: Journ. of Biol. Chem., 3 (1907).

ments on the influence of different stimuli on the amount of enzyme have been carried out only on dogs, but these animals are not suitable for such investigations.

The action of ptyalin begins in the mouth during mastication, and is continued in the stomach. The action is checked by acid, but the gastric juice comes in contact only with the outer parts of the contents of the cardiac end. It has been pointed out by Ellenberger,[1] Cannon,[2] and Grützner,[3] that the inside of the food-mass lying in the cardiac end of the stomach has the slight alkaline reaction of the saliva which moistens the food. Thus under normal conditions a great deal of starch is dissolved in the stomach, and reaches the intestine as dextrin or maltose. Here it meets with the pancreatic juice, which contains the same ptyalin as the saliva. The pancreatic juice and pancreatic extract are rich in ptyalin in all vertebrates which have been examined; in man and other mammals ptyalin is present during the last few weeks before birth.

We know of a variety of stimulants for the secretion of the pancreas, which induce the secretion of juices of different properties.

1. The presence of acid in the intestine calls forth, by means of a hormon *secretin* of the intestinal wall, a strongly alkaline juice poor in solids, especially proteins.

2. Odor, sight, or the taste of food, provokes, by a

[1] Ellenberger: Pflüger's Arch., 114 (1906).
[2] W. B. Cannon: Amer. Journ. of Physiology, 9 (1903).
[3] P Grützner: Pflüger's Arch., 106, 463 (1905).

nervous mechanism, the so-called psychical reaction, a secretion containing much protein, but little alkali.

3. Fats, fatty acids, and soaps provoke a secretion from the duodenum by an unknown mechanism. This juice is still richer in proteins than that occasioned by the psychical reaction, and is only slightly alkaline.[1] Dogs fed with meat secrete a pancreatic juice resembling the pure secretin-juice, and this is, according to Walther,[2] a pupil of Pawlow, poor in ptyalin. Dogs fed with milk and bread secrete by a summation of stimuli a more concentrated juice, which contains more ptyalin. We have no evidence of other adaptations of the enzymes of the pancreas to the properties of food.

Enzymes inverting disaccharides are limited to the small intestine. It is pointed out by Bainbridge,[3] Plimmer,[4] and Ibrahim[5] that the pancreas does not produce lactase even in new-born mammals fed exclusively upon milk. Maltase occurs in all tissues, while lactase and invertin are produced exclusively by the mucous membrane of the small intestine. Cane sugar and milk sugar can, therefore, be utilized only if they meet with the enzymes of the intestine. As was said, enzymes probably attack disaccharides not only in the lumen of the intestine, but to a great extent during the passage through the cells.

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[1] J. P. Pawlow: Nagel's "Handbuch der Physiologie," Bd. ii., 1906.

[2] A. A. Walther: Arch. des Sciences biolog. de St. Pétersbourg, 7(1899).

[3] F. A. Bainbridge: Journ. of Physiol., 31 (1904).

[4] R. H. A. Plimmer: *ibid.*, 34 (1906).

[5] J. Ibrahim and L. Kaumheimer: Zeitschr. f. physiolog. Chem., 62 (1909).

Maltase is always found in the extract of the intestinal mucous membrane, and invertin or sucrase is one of the enzymes occurring first in embryonic life. In the fetus of man, dogs, and cats, it can be found earlier than erepsin, trypsin, and ptyalin.[1] We do not yet understand what this fact means; it is remarkable that we find in the stomach and in the intestine of the fetus a reducing and levorotatory fluid. Mendel[2] found invertin in pigs only shortly before birth, and maltase and lactase in the middle of fetal life. The human fetus produces lactase only shortly before birth.[3]

Of the inverting enzymes the most interesting to-day is lactase, because lactase is the only enzyme for which we seem to have evidence of adaptation to diet, *i.e.*, to the need of the body during individual life. Milk sugar occurs only in the milk of mammals, and it has been pointed out in recent years by Weinland,[4] Bainbridge, Plimmer, Mendel,[1] and others that all young, sucking mammals produce lactase, while birds, frogs, and invertebrates do not. The presence of lactase in adult mammals is questioned. Weinland, Mendel, Bierry, and others have come to the conclusion that, as a rule, lactase is not found in adult animals, such as the pig, guinea-pig, rabbit, dog, sheep, cat, ox, or horse. Weinland observed, however, that dogs fed for some weeks with milk, or milk sugar,

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[1] J. Ibrahim: Gesellsch. f. Kinderheilkunde, 1908.

[2] L. B. Mendel and P. H. Mitchell: Amer. Journ. of Physiol., 20 (1907).

[3] J. Ibrahim: *loc. cit.*

[4] E. Weinland. Zeitschr. f. Biol., 38 (1899).

produce lactase again, and he concluded that this was adaptation to diet. Further experiments carried out by Plimmer seem to show that no adaptation occurs. He always found lactase in the intestines of adult pigs, dogs, and cats, and failed to find it in adult guinea-pigs, whether fed with milk or not. These discrepancies are without doubt occasioned by the great difficulties experienced in detecting and estimating galactose and glucose, besides the milk sugar, and by the danger that, in extracts of the intestine crowded with bacteria, glucose is formed by bacteria and not by the enzyme. In my opinion, the results of Weinland and Mendel are not set aside by Plimmer. The occurrence of lactase induced by feeding milk sugar is the only case of an adaptation of enzymes during individual life.

At this point may be mentioned the experiments [1] carried out with the aim of seeing whether animals can produce new enzymes not natural to them. The results were negative. Birds fed with milk sugar do not produce lactase, nor do dogs fed with inulin [2] or polysaccharides built up from mannose and galactose, produce the corresponding enzymes which occur in plants and bacteria.

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[1] E. Weinland: Zeitschr. f. Biol., 47 (1905).

[2] L. B. Mendel and T. Taiki: Journ. of Biolog. Chemistry, 2 (1906).

CHAPTER XII

The Lipases or Steapsins of the Alimentary Canal

The three known lipases or steapsins work in the same way; *i.e.*, they split neutral fats into fatty acids and glycerin. They differ only in the conditions of action, the final reaction, the activation by bile, and such secondary characteristics. The lipase of the stomach was discovered by Volhard [1] in 1900. It is secreted by the cells of the gastric glands, and is found both in the natural gastric juice, in the contents of the stomach, and in the extracts of the glands. Because oil can produce under special conditions the entrance of bile and pancreatic juice into the stomach, some authors have believed that Volhard's lipase is the pancreatic lipase present in the stomach, but Volhard and Magnus and Laqueur [2] have found it in the juice of Pawlow's so-called little stomach, separated completely from the gastric cavity, and inaccessible to the pancreatic juice. Whether gastric juice always contains lipase, or whether the secretion occurs only in response to a special stimulus, has not been investigated. The gastric lipase is active in a slightly acid medium; a stronger acid reaction

[1] F. Volhard: Münchener med. Wochenschrift, 1900, 141 and 195.—Zeitschrift f. klin. Medizin, 42, 414; 43, 397 (1901).—Hofmeister's Beiträge, 3 (1902); 7 (1905).

[2] E. Laqueur: Hofmeister's Beiträge, 8 (1906)

checks its action. It is to be noted that fats retard the secretion of gastric juice and thus lessen the amount of acid in the stomach. The gastric lipase attacks only emulsified fats, for instance the fats of milk, and especially of eggs. For estimating gastric lipase, Volhard uses egg yolk. There is no evidence of the existence of a zymogen or an activator. The new-born child produces gastric lipase.[1]

The pancreatic lipase has been known for a long time. It attacks all fats, whether emulsified or not. It works best in neutral or slightly alkaline reaction. It is more unstable than other lipases, and perishes rapidly in natural pancreatic juice or fresh water-extracts of the gland; it is apparently destroyed by trypsin. Pancreatic juice always contains a certain amount of ready lipase. Most of it occurs as a zymogen, which is made active by bile salts; the only activation completely explained. Fats stimulating the intestinal wall cause pancreatic juice and bile to be poured out at the same time. The action of the pancreatic steapsin is promoted by the bile salts in two ways. The enzyme is activated by them, and they dissolve the fatty acids set free by the steapsin and enable them to be absorbed.[2]

The third lipase occurring in the enteric juice was discovered by Pawlow and Boldyreff.[3] It attacks only

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[1] J. Ibrahim and T. Kopez: Zeitschr. f. Biol., 53 (1908).

[2] B. Moore and D. P. Rockwood: Journ. of Physiol., 21 (1897).

[3] W. Boldyreff: Arch. des Sciences biolog. de St. Pétersbourg, 11 (1904).

emulsified fats. The optimum for its action is a slightly alkaline reaction. It is not activated by bile.

There can be no doubt that all fats are split by these three enzymes and dissolved in water through the agency of bile. During passage through the cells of the mucous membrane, neutral fats are again produced from the dissociation products. Lipases can both build up and split fats. It depends upon the concentration and upon the presence of more or less water, as to which process prevails. The synthesis of fats in the cells lining the intestine is due to the lipase manufactured by these cells. As we can see microscopically, very minute drops of fats or of fatty acids (which cannot be distinguished optically from each other) become visible in the first row of epithelial cells. During the solution of these acids in the watery contents of the intestine, we do not see them. On entering the cells, they are precipitated, that is to say, they are separated from the water and then the synthetic action of the intestinal lipase begins. After synthesis, fats lose their solubility in water, and, as insoluble globules, they cannot pass the wall of the endothelial cells and enter the capillaries, but must follow the spaces of the reticular tissue. They are therefore conveyed into the lacteal radicles and the lymphatic vessels.

CHAPTER XIII

Proteolytic Enzymes

The proteolytic enzymes, pepsin, trypsin, and erepsin, are exactly adapted to one another. The proteins are handed from one enzyme to the next like bricks in the hands of the mason. The first passes the brick to the second, the second to the third, etc. But while the brick goes from hand to hand without changing, the proteins are divided into small pieces before being useful to build a new body. The first enzyme, pepsin, dissolves all natural proteins, but converts them only into proteoses and peptones; the second, trypsin, does not dissolve all, but most proteins, and splits them and the peptones chiefly into amino-acids; erepsin does not act upon any natural protein, and digests proteoses but slowly, but it dissociates the peptones formed by pepsin much more rapidly than does trypsin; it converts peptones completely into amino-acids. Thus the complete disintegration of the protein molecule is secured, even if gastric digestion or the secretion of the pancreatic juice is somewhat disturbed. We know in human pathology cases of the so-called gastric achylia, and I have observed a similar condition in the dog,[1] in which the secretion of the stomach may fail com-

[1] O. Cohnheim: Münchener med. Woch., 1907.

pletely without causing any trouble to the patient, and without reducing the availability of food.

Pepsin acts in the presence of hydrochloric acid. Kühne [1] was the first to observe that pepsin does not form amino-acids, but only peptones. His view has been supported in recent years by many observers.[2] No free tyrosine or tryptophane occurs in the stomach or in peptic digestion. Tyrosine is nearly insoluble in water, and the so-called tryptophane reaction, a violet color with bromine or chlorine water and acetic acid, is given only by the free tryptophane not bound in the protein molecule. Thus the absence of tyrosine crystals and the negative result of the tryptophane reaction distinguish peptic from tryptic and other digestions. Pepsin does not directly produce the so-called peptones, but the transition is a gradual one, and the intermediate products were called albumoses by Kühne.[3] Kühne and most earlier investigators, studying peptic digestion *in vitro* or in the stomach, believed that the proteoses were the chief products of pepsin digestion. Now we know from Tobler that in the living stomach peptic digestion proceeds much more rapidly, and that proteoses can be found early in very small quantity in the chyme leaving the stomach. Even in artificial digestion, proteoses disappear rapidly as de-

[1] W. Kühne: "Untersuch. a. d. physiol. Institut Heidelberg," ii. (1878).—Verhandl. des naturh. Vereins Heidelberg (N. F.), i.

[2] L. Tobler: Zeitschr. f. physiolog. Chemie, 45 (1905).—S. S. Salaskin, *ibid.*, 32 (1901); 38 (1903).—O. Cohnheim: Münchener med. Woch., 1907.

[3] The term proteose, as suggested by Chittenden, will be used by the editor instead of albumose.

scribed, if we use dialysis and thus imitate natural conditions. The proteoses seemed, for many years, to be substances of the highest interest, and their occurrence, their absorption, their classification, and their toxicity, were often and thoroughly studied; we must now say, however, that their importance was much exaggerated. Of the peptones formed by pepsin, no chemical individuals seem as yet to have been isolated. Most of them, but apparently not all, give a positive, strong, biuret reaction.

The secretion of gastric juice can be stimulated both by nervous mechanisms from the sense-organs of the head and from the intestine, and by a chemical mechanism, by a hormon produced in the antrum pylori. These juices contain, according to Pawlow, the same quantity of pepsin, but if we add starch to meat, stimulating secretion, the concentration of pepsin rises. The function of this action has not been explained, but it is remarkable that many years ago Schiff [1] described peptogenic substances, that is to say, substances which, without causing secretion, give rise to the production of pepsin in cells.

The amount of hydrochloric acid seems to be identical in man, dog, and goat, but human juice yields less pepsin than the dog's secretion, and thus dissolves less protein. Ten cc. of the contents of the stomach three hours after a test meal, contains 12 mgm. nitrogen in the case of man, and 30 mgm. in the case of the dog.[2]

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[1] A. Herzen: Pflüger's Arch., 84 (1901).

[2] O. Cohnheim and G. L. Dreyfus: Zeitschr. f. physiol. Chemie, 58 (1908).

All proteins are dissolved in the gastric juice, and the casein of milk becomes solid in the stomach. It has been known for a long time, that an extract of the calf's stomach, called rennet, has a remarkable effect in rapidly curdling milk, and this property has been utilized for thousands of years in the manufacture of cheese. If a few drops of gastric juice or of gastric extract are added to milk, and the mixture exposed to body temperature, the milk curdles into a complete clot in less than a minute. If the action is continued, the clot is dissolved. In 1872, Hammarsten showed that the curdling is due to the precipitation of casein, and that the curdling properties of the gastric infusions are destroyed by boiling. He suggested that in curdling the casein is converted into a new compound, paracasein, insoluble in water, and that this conversion is caused by a special enzyme, rennin. This view has been generally accepted for over thirty years, although we to-day have reasons for doubt. Casein is the only protein precipitated by rennin, and occurs only in mammals' milk; nevertheless, the alimentary canal or the tissues of birds, frogs, fish, invertebrates, bacteria, and plants, all contain considerable rennin. I remember how astonished I was to meet with rennin in polyps, sea-urchins, and star-fish, which never drink milk. Pawlow[1] solved the riddle; he found that rennin and pepsin cannot be separated from each other and that the amount of rennin and pepsin in

[1] J. P. Pawlow and S. Parastschuk: Zeitschr. f. physiolog. Chem., 42 (1904).—W. W. Sawitsch: *ibid.*, 55 (1908); 63 (1909).—M. Jacoby: Biochem. Zeitschr., 1 (1906).

gastric juices, gastric extract, and dried enzyme powder is always proportional. Vernon [1] noted the uniformity of rennin and trypsin in pancreatic extracts, and Pawlow and his collaborators, especially Sawitsch, were able to refute all objections. We cannot doubt to-day the identity of pepsin and rennin, but I think that we must go further than Pawlow, who believed that the same enzyme-molecule has two enzymotic actions. Hammarsten observed that, after curdling, not all the casein is removed from the solution, but that there remains a remnant of peptone-like substance; and he suggested that, in curdling, the casein is split into two compounds. Because all proteolytic enzymes affect casein in the same way as pepsin does, it seems to me that the best explanation for all the facts is that the curdling is only the first step toward the dissociation of casein. Rennin is therefore not a special enzyme, but the casein is disintegrated very easily by all proteolytic enzymes, and the first cleavage product, the first proteose of the casein, is insoluble in acids and in water containing lime salts. The misleading phenomenon is due to the extreme sensitiveness of the clotting reaction, which led to a separation of the clotting of casein from the common action of pepsin. Casein is the most easily digested of all proteins; it is itself acid and needs no further acid, and the precipitation of casein is more obvious than the beginning digestion of other proteins.

[1] H. M. Vernon: Journ. of Physiol., 28 (1903).

The stomach, in mammals, is not peculiarly adapted to the needs of milk digestion, but the mammary gland secretes a protein adapted in a remarkable way for the processes occurring in the stomach. Fluids pass rapidly through the stomach without being digested.[1] The milk, so rich in proteins and fats, must be thoroughly digested, and this effect is obtained by changing milk to a solid mass. Tobler[2] observed that, in the stomach, milk is divided into two parts. Water and milk sugar leave the stomach in a short time, while casein and fats remain for many hours in the stomach and are digested there slowly and completely. Clotting of milk is very important, but rennet is no special enzyme; and this fact is of great interest, because many investigations of the general nature of enzymes and of the laws of enzyme-action, deal with rennet. If clotting is only a short intermediate process, such observations lose value as evidence, and we meet here with one of the unfortunate cases in which science in stepping forward obliterates and renders useless the hard and skilful work of a whole generation of prominent men. The clotting of milk and the properties of the serum have been studied by biologists and chemists like Hammarsten, and now his work is set aside. Just as casein gives rise to an insoluble proteose, so do occasionally other proteins. We can see a precipitation more or less bulky, particularly on heating proteins with proportionately small quantities of pepsin and an insufficient quantity of

[1] O. Cohnheim: Münchener med. Wochensch., 1907.

[2] L. Tobler: Gesellsch. f. Kinderheilkunde, vol. 23 (1907).

acid, and if, after a time, fresh pepsin is added. Danilewsky [1] and his collaborators, Sawjalow, Kurajeff, and others, have thought that they were dealing with a synthetic process of great physiological importance, the building up of protein from peptones. They gave to the substance precipitated the name *plastein*. Salaskin, Bayer, Levene,[2] and others have shown that plastein is not a protein, but a proteose-like body or an impurity, precipitated by the acid or one of the substances of the extract. It most resembles the hetero-proteose or dys-proteose of Kühne which becomes insoluble during peptic digestion. If the digestion continues, the plastein is dissolved again and finally is converted into peptones like other proteoses. The gastric contents which pour through the pylorus contain no plastein, and no conclusive evidence has been brought forward that the protein absorbed from the stomach yields any of this plastein.

The stomach, or at least a part of the stomach, contains a second proteolytic, or better, peptolytic, enzyme, observed by Malfatti,[3] Bergmann,[4] Takamura,[5] and others.[6] The enzyme, or as most authors say, the protease, like

[1] Okunew: Diss. St. Pétersbourg, 1895.—Sawjalow: Pflüger's Arch., 85 (1901).—Kurajeff: Hofmeister's Beitr., 1 (1901).—Nürnberg: *ibid.*, 4 (1903).—Bayer: *ibid.*, 4 (1903).—Salaskin: Zeitschr. f. physiolog. Chem., 36 (1902).

[2] P. A. Levene and van Slyke: Biochem. Zeitschr., 13 (1908); 16 (1909).

[3] H. Malfatti: Zeitschr. f. physiolog. Chem., 31 (1900).

[4] P. Bergmann: Skandinav. Arch. f. Physiolog., 18 (1906).

[5] Takamura: Zeitschr. f. physiolog. Chem., 63 (1909).

[6] O. Cohnheim: Münchener med. Wochenschr., 1907.

erepsin, does not dissolve protein, but splits peptones and converts them into amino-acids. It is not secreted, and seems to be absent from the mucous membrane of the cardiac end of the stomach. It is found in extracts of the thick mucous membrane of the antrum pylori, or the pyloric end. One might think that we were dealing with an autolytic enzyme, or tissue enzyme produced for the metabolism of the tissue (see below). But the protease or the erepsin works in acid solution of such strength as prevails in the contents leaving the pylorus, and it is rather probable it attacks the peptones formed by the pepsin, and is absorbed in the stomach. Moritz, v. Mering, and others had observed that pure water or dilute solutions of sugar and salts run rapidly through the stomach without being absorbed in this way. But I was able to demonstrate [1] that such fluids run along a special channel from the cardiac end to the pylorus—a channel to which Waldeyer has given the name "magen-strasse." They are not comparable with the real chyme formed in the stomach by the digestion of food. As Tobler [2] has pointed out, by means of a really natural method, thirty per cent of the nitrogen of the meat disappears in the stomach of the dog. In the cardiac end, the mucous membrane is almost wholly composed of glands, and between them are left only very small ridges covered with mucous cells, which can scarcely absorb large quantities. The chief absorption must occur in the antrum pylori

[1] O. Cohnheim: Münchener med. Woch., 1907.

[2] L. Tobler: Zeitschr. f. physiolog. Chem., 45 (1905).

with its glands less closely packed, and the hydrochloric peptones are split into amino-acids by the protease, before they pass into the mucous membrane. Under pathological conditions, if the epithelial layer is injured, the protease can be found in the contents of the stomach. The presence of amino-acids in the stomach is used as a diagnostic method which demonstrates injuries of the epithelial layer by ulcer or cancer.

The trypsin dissolves most natural proteins, with two exceptions. First, the genuine colloidal albumins, as serum albumin or egg albumin are scarcely or not affected at all by trypsin; raw, fluid egg-white can run rapidly through the stomach, and then it is not digested, but is absorbed unchanged, and eliminated with the urine. Secondly, the collagen of connective tissue, as was first noted by Kühne and Ewald,[1] is not dissolved by trypsin. This exception is used by Adolf Schmidt[2] who gives to a patient raw meat; collagen-fibres are left untouched by trypsin, and their occurrence in the feces, which can readily be observed microscopically, is an evidence of the failure of pepsin action.

As for the other proteins, if the whole alimentary canal and reflexes are healthy, the lack of one enzyme or of one digestive juice can be compensated for; the chief regulation is apparently the rhythmic segmentations described by

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[1] Kühne and Ewald: Heidelberger Natur. med. Verein, N. F., i. (1876).—A. Ewald: Zeitschr. f. Biol., 26 (1890).

[2] Adolf Schmidt: "Probekost," Wiesbaden, 1904.—R. Baumstark and O. Cohnheim: Zeitschr. f. physiol. Chem., 65 (1910).

Cannon, by which the small intestine squeezes out the chyme until all digestible substances are brought into solution and absorbed. The capacity of compensation assures the safety of the organism, but it makes physiological investigations difficult. We can deprive a dog of the whole stomach, and yet the animal can digest very well; apparently the stomach is not necessary for life. If we now extirpate the pancreas, the dog will die. On the contrary, when we deprive a dog of the pancreas, we cause but few disturbances of digestion; the pancreas is not necessary for life. When we now extirpate the stomach, however, the dog dies.

Proteins dissolved by trypsin are converted first into peptones, which, however, clearly differ from pepsin peptones. Afterward, they are converted to a great extent into amino-acids. Whether all amino-acids must be derived from peptones, is not certain. The question involves knowledge of the configuration of the protein molecule, and especially of the different linkings of the amino-acids, and I cannot discuss this fully. We may, however, note the following facts: Immediately after the beginning of the action of trypsin, amino-acids are split off, while the rest of the protein is still untouched. This residue gradually becomes smaller, but the whole molecule is never disintegrated. The remainder contains phenylalanine, proline, glycocoll, and glutaminic acid, while tyrosine, tryptophane, and cystine are set free completely by trypsin. Leucine, lysine, arginine, and aspartic acid are partly liberated. Like natural proteins, peptones

formed by peptic digestion are partly dissociated by trypsin; they give rise both to tyrosine and other amino-acids, and to peptones which vigorously resist further dissociation by means of trypsin. The remainder, which is not acted upon, Kühne called the "antigroup." We do not know whether the amino-acids are linked here in a different manner, or what the reason may be. The artificial peptids, di- and poly-peptids, built up by E. Fischer, are also only partly dissociated by trypsin. It is impossible now to foretell whether a peptid is touched or not, or to tell why alanylglycine is dissociated, and glycylglycine not, why tetraglycylglycine is dissociated, and triglycylglycine not, etc. Physiologically, the differences have no weight, because erepsin dissociates all peptones and all artificial peptids. Thus the peptones formed by trypsin are always intermediate compounds, and disappear after a short time. For the chemistry of enzymes and of proteins, these observations may become very important. At present they are isolated facts, which do not lend themselves to explanation.

Trypsin is secreted as zymogen, and is completed or activated by enterokinase. This holds good, however, only for the pancreatic juice secreted after the stimulus by secretin. The pancreatic juice secreted after nervous stimulus, and after the stimulation of the intestine by fats or soaps, contains, besides trypsinogen, ready efficient trypsin. The juice secreted by meat is an almost pure secretin-juice, and contains no trypsin or but very little, but dogs eating a mixed food secrete a juice which con-

tains both. Discrepancies among authors are apparently due to these differences in the secretion under different conditions, but these differences have led to an observation of very great importance. Accordingly as dogs are fed on a meat diet or a meatless diet, they secrete different pancreatic juices, and if we suddenly change the diet, the juice is not changed suddenly, but only gradually during many days, so that during the interval digestion may be disturbed. So far as I can see, it is the first experimental observation which explains the great influence of alteration of diet on man.

For other adaptations there is no evidence. Like ptyalin—see above—the amount of trypsin seems to differ in juices provoked by different stimuli. But a special adaptation of trypsin in case of need, according to the amount of proteins or the digestibility of proteins, has not been demonstrated.[1]

Some authors have thought that trypsin is not one enzyme, but a group of proteolytic enzymes, one of which attacks casein, one gelatin, one fibrin, etc. I do not think that the experiments [2] which are cited are conclusive. If a weak trypsin solution digests casein at 37° C., and does not digest gelatin at 23° C., the difference does not allow the suggestion of two enzymes.

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[1] J. P. Pawlow: Nagel's "Handbuch d. Physiologie," ii.

[2] O. Cohnheim: Zeitschr. f. physiolog. Chem., 33 (1901); 35, 36 (1902); 47, 49 (1906); 51, 52 (1907).—S. S. Salaskin: *ibid.*, 35 (1902).—F. Kutscher and J. Seeman: *ibid.*, 35 (1902).—J. H. Hamburger and J. Hekma: Akad. v. Wetenschappen, Amsterdam, 1902.

Like other proteolytic enzymes, trypsin provokes curdling of milk, and dissolves again the clotted casein. Because efficient trypsin redissolves casein very rapidly, pancreatic rennin has been less studied than gastric rennin.

The last proteolytic enzymes to be mentioned are those of the small intestine,—erepsin and arginase.

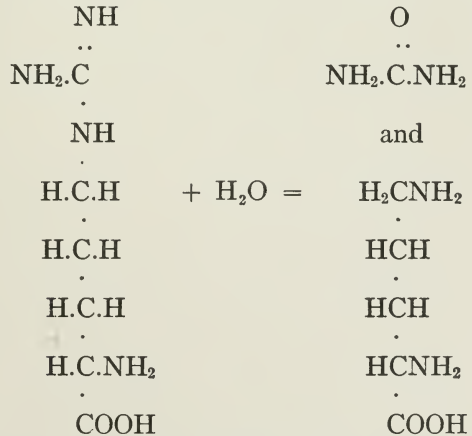
Erepsin is found in the enteric juice, and can always be extracted in great amount from the intestinal mucous membrane ground with sand. It disintegrates all peptones and converts them into amino-acids, and it also disintegrates all artificial peptids.[1] In experiments on the efficiency of ereptic dissociation, it is necessary to work with the compounds which are naturally acted upon by erepsin. Proteoses are converted by erepsin; but it is quite well known that they reach the intestine in but very small amount. The designation "peptone," applied to many purchaseable products which yield chiefly proteoses, has resulted in much error. The peptones formed by natural peptic digestion are rapidly converted by erepsin. The mass of meat-peptones that leaves the dog's stomach in three hours, is split by the extract of a dog's intestine in two hours. The extract of the intestines of two dogs completely converts in three hours 30 gm. of peptones formed in peptic digestion. One-fourth of the extract of one intestine converts 7 gm. in two hours. In enzyme-investigations, many authors have forgotten that a man metabolizes 100 gm. of protein per day, and that

[1] E. Abderhalden and Y. Teruuchi: Zeitschr. f. physiolog. Chem., 49 (1906).

an enzyme which digests 1 gm. of fibrin in 12 hours, or the proteins of a small organ in four months, cannot be a true digestive enzyme, as erepsin is. Among the natural complex proteins, protamine is split by erepsin, histone and casein are slowly and partly converted, and all other proteins are left untouched. That histones are attacked by erepsin, may be of some importance, because most probably all tissues yield histones, and the so-called autolytic enzymes resemble erepsin perhaps more than trypsin.

Erepsin is found in the human fetus in the seventh month.[1] It can also be found in the feces.[2]

Arginase, found in the liver and intestine by Kossel and Dakin, splits arginine into ornithine and urea:



[1] E. Jaeggy: Zentralbl. f. Gynäk., 1907.—L. Langstein and M. Soldin, Jahrbuch f. Kinderheilkunde, 67 (1908).—D. E. Edsall: Journ. Amer. Med. Association, 1907.

[2] A. Schittenhelm and Fr. Frank: Zeitschr. f. experim. Path. u. Therap., 8 (1910).

It completes the action of erepsin, loosening the second linking of amino-acids in the protein-molecule, besides the peptid linking.

The occurrence of arginase in the enteric juice has not been investigated, but the extract contains it in much smaller amount than the liver extract, so that it is not certain whether we have in arginase a digestive or a so-called autolytic enzyme. Perhaps arginase has a function in the particular metabolism of the intestine and liver, perhaps, like the peptolytic enzyme of the stomach, it may attack arginine in its course of absorption. So long as we do not know the fate of the absorbed protein or the intermediate metabolism of proteins, the question cannot be answered with certainty.

CHAPTER XIV

Miscellaneous and Vegetable Enzymes

Besides carbohydrates, fats, and proteins, food contains other compounds, such as lecithin, nucleic acid, and cholesterin, which are disintegrated in the alimentary canal.

Nucleic acid becomes insoluble in the stomach,[1] because it forms salts with proteins and proteoses which are insoluble in an acid medium. This precipitate was called "nuclein" by earlier authors, and the undissolved remnant of peptic digestions of meat or tissues is mainly this nuclein. This remnant is gradually dissolved, and the nucleic acid is absorbed in the small intestine.[2] It is possible that there is a simple solution of nucleic acid in the alkaline pancreatic juice, and that portions of the nucleic acid can pass the epithelial cell and enter the blood without dissociating. Probably it is converted in the lumen of the intestine, or in the course of absorption. Kutscher[3] allowed pancreas and mixtures of pancreas

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[1] F. Umber: Zeitschr. f. klin. Med., 43 (1901).

[2] Gumlich: Zeitschr. f. physiolog. Chem., 18 (1893).—T. H. Milroy: *ibid.*, 22 (1896).—P. M. Popoff: *ibid.*, 18 (1893).—E. Abderhalden and A. Schittenhelm: *ibid.*, 48 (1906).—T. Araki: *ibid.*, 38 (1903).—F. Sachs: *ibid.*, 46 (1905).—M. Nakayama: *ibid.*, 41 (1904).

[3] F. Kutscher: Zeitschr. f. physiolog. Chem., 32 (1900); 39 (1903); 44 (1905).

and intestine to stand for a long time at the temperature of the body. As a result, he found, besides the cleavage products of proteins, purine bases and pyrimidines, which are the well-known dissociation products of nucleic acid. Therefore, pancreas and intestine must contain nuclease, and Sachs, Nakayama, and others have made extracts of the gland and the mucous membrane, and have studied their action on nucleic acid or nucleic salts. The nucleic acid was converted into the co-called nucleic acid-*b*, a compound of different physical properties; the further conversion into purine bases, pyrimidines, and phosphoric acid could be observed only in small amount, because nuclease loses its activity very rapidly in solutions containing trypsin or other proteolytic enzymes. But I think that conversion would proceed more readily under natural conditions, and that nucleic acid is split to a great extent during absorption.

The precipitation of nucleic acid in the stomach and its solution by pancreatic nuclease have been used by Adolf Schmidt as a clinical method. Schmidt observed that, under normal conditions, nuclei, for instance of the calf's thymus, disappear in the intestine, but he was able to find nuclei in the feces of pathological cases. He made the inference that in these cases pancreatic secretion is checked. If he is right, intestinal nuclease would be no digestive enzyme. The occurrence of nuclease in all tissues prevents us here from drawing a clear conclusion.

Lecithin resembles fats in chemical configuration and physical behavior, solubility, and most properties. Like

fats, it consists of glycerin-esters of fatty acids. Lecithin differs from fat in the further combination of glycerin with phosphoric acid and the base choline. It is an essential part of every living cell, therefore food nearly always contains lecithin. Kutscher and Lohmann,[1] and Bergell, demonstrated that the pancreas and the intestine yield an enzyme which disintegrates lecithin. They allowed pancreas and intestine to stand for several months and digest its own substance, and they then found choline in large quantities. We have no further evidence for the secretion of this enzyme, but it is probable that lecithin is digested in the intestine, and it is also probable that lipases dissociating other esters attack lecithin as well.[2]

Animal food often contains hæmoglobin and its non-protein constituent, hæmatin. Hæmoglobin is disintegrated by the acid of gastric juice,[3] and hæmatin must be present everywhere in the intestine, because it contains iron in non-ionic form, and, according to the microscopical studies of Quincke and Hochbauer,[4] iron can be found in ionic form in the absorbing cells of the duodenum and in the upper small intestine. The enzyme is unknown.

Cholesterin seems to be transformed only by bacteria,

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[1] F. Kutscher and Lohmann: Zeitschr. f. physiolog. Chem., 39 (1903); 41 (1904).

[2] C. Schumoff-Simanowski and N. Sieber: Zeitschr. f. physiolog. Chem., 49 (1906).

[3] R. v. Zeynek: *ibid.*, 30 (1899).

[4] H. Hochbauer and H. Quincke: Arch. f. exper. Path. u. Pharmak., 37 (1896).—E. Abderhalden: Zeitschr. f. Biol., 39 (1900).—A. Hofmann: Virchow's Arch., 151 (1898).

and for many years it has been the general opinion that the cellulose of vegetable food is also dissolved and dissociated by the bacteria of the alimentary canal. No one has observed any influence of any digestive juice or extract of digestive organs upon cellulose, and this indigestibility is a matter of great biological importance. Nevertheless, cellulose disappears in passing through the alimentary canal of herbivorous animals, and even in man, some is digested.[1] The action of micro-organisms takes place in the large intestine, and in the ruminants in the first stomach. This action has been studied *in vitro* by Tappeiner.[2] In human pathology, it was found by Adolf Schmidt[3] that the amount of cellulose which disappears in passing through the alimentary canal of man varies greatly, but without any evidence for differences in the quantity or kind of bacteria. It is not impossible that for the disintegration of cellulose a co-operation of bacteria and the living cell wall of the intestine is necessary. For the part played by the living wall I have three examples:

1. As Schütz[4] has pointed out, if we introduce a great quantity of bacteria, such as Bacillus pyocyaneus, through a fistula into the small intestine of a dog or cat, the bacteria are killed in a very short time. The surviving intestine removed from the body and kept in sterilized blood, acts

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[1] W. v. Knieriem: Zeitschr. f. Biol., 21 (1885).

[2] H. Tappeiner: *ibid.*, 19, 20 (1884).

[3] H. Lohrisch: Zeitschr. f. physiolog. Chem., 47 (1906).

[4] R. Schütz: Arch. f. Verdauungskrankheiten, 7 (1901).—Verh. des Kongresses f. innere Medizin, 1909.

similarly. But the surviving organ loses its power thirty minutes after removal from the body, at the same time that the epithelial cell loses its absorbing power. Neither the dead nor the dying cells, nor extracts of the mucous membrane, nor any secretion, can kill bacteria, but only the living cell with its structures can do so.

2. A lower marine invertebrate, Actinia,[1] the well-known sea anemone, has often been investigated, because it can digest great quantities of meat, fibrin, or other proteins without secreting any digestive fluid. According to the observations of many authors, Chapeavese, Mesnil, and others, the mesenteric filaments, the digestive organs of these animals, can dissolve and dissociate protein, but only when they are in immediate contact with it.

3. The surviving wall of the intestine converts peptones in another way than do the extracts of the mucous membrane.

It is possible that, in the stomach of living ruminants, and in the cecum and the large intestine of man and herbivora, bacteria find conditions so much better than we can imitate, that they dissolve cellulose in much greater quantity than in Tappeiner's experiments. But it is also possible that they carry on the digestion of cellulose in a manner unknown at the present time.

Through the whole series of invertebrates we find no difference in the occurrence and arrangement of the digestive enzymes, except in the cases of lactase and

[1] O. and R. Herting: "Die Aktinien," Jena, 1879.

salivary ptyalin, already mentioned. The pancreatic ferments, gastric pepsin and intestinal erepsin and invertin, seem to be identical in mammals, birds, frogs, and fish. And so does the association between the intestine and pancreas, or intestine and stomach. The digestive organs of invertebrates show many differences in arrangement and development, according to the biological need of the species. But their tools are the same enzymes described above in mammals, namely, ptyalin, maltase, invertin, steapsin, and nuclease. Only, instead of the three proteolytic enzymes adapted to one another, we find one enzyme which dissolves proteins and, at the same time, converts them, partially or completely, into amino-acids as thoroughly as do trypsin and erepsin. Most of these enzymes work in a neutral reaction, some are assisted by an acid reaction, like pepsin, and split proteins completely.[1] In one of the large higher invertebrates, the Octopus vulgaris, a cephalopod, it was possible to show that the secretion of enzyme was governed by the nervous system.[2] In actinia and some other lower invertebrates, it seems that enzymes act only in vacuoles of the protoplasm, and are not excreted from the cells. In these cases digestible substances are surrounded by moving protoplasm and digested in the space thus formed. In protozoa this kind of digestion has been observed by Nierenstein,[3]

[1] L. B. Mendel and H. C. Bradley: Amer. Journ. of Physiol., 13 (1905).

[2] O. Cohnheim: Zeitschr. f. physiolog. Chem., 35 (1902).

[3] E. Nierenstein: Zeitschr. f. allgem. Physiolog., 5 (1905).

who saw that digestible substances were brought into a vacuole, that acid and enzyme were secreted into the vacuole, and that proteins were thus dissolved. Digestion occurs inside of the cell, but outside of the protoplasm.

Bacteria yield all types of enzymes: sucroclastic enzymes, lipases or steapsins, nucleases,[1] lecithases, and proteolytic enzymes. In some cases, it has been possible to extract the enzymes from the bacteria and investigate them in solution. In most cases only the action of the bacteria themselves upon proteins or sugars has been observed, and conclusions have been drawn as to the existence of corresponding ferments in the bacteria. The proteolytic enzymes of yeast [2] and other micro-organisms closely resemble trypsin, and have received the name "endotrypsin." They give rise to the ordinary amino-acids, but in such experiments the amino-acids are often converted into acids, keto-acids, alcohols, etc., because in these unicellular organisms digestive enzymes and metabolism-enzymes cannot be separated (see below).

Among plants, full-grown individuals have no special digestive organs like animals, but in very young plants the germ is surrounded by storage-substances which are used for food in growth; and these substances, starch, fat, and proteins, are digested or dissolved by enzymes secreted by the growing germ. Therefore germinating

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[1] F. Kutscher: Zeitschr. f. physiolog. Chem., 32 (1900); 39 (1903).

[2] M. Hahn and Geret: Zeitschr. f. Biol., 40 (1900).—F. Kutscher: Zeitschr. f. physiolog. Chem., 32 (1900); 34 (1902).—E. Salkowski: *ibid.*, 13 (1889).

seeds yield powerful enzymes, which act outside the protoplasm, and can easily be obtained and purified. The diastase of seeds is one of the longest known enzymes: the steapsins of the seeds of the castor-oil plant are used in the manufacture of soaps from oils.[1] I have mentioned that this steapsin acts well only in an acid medium, and that this acid is produced by converting the carbohydrates of the seed into organic acid at the time of germination. The proteolytic enzymes of germinating seeds have been thoroughly studied by E. Schulze[2] in Zurich. Under normal conditions, the cleavage products formed by these enzymes are used for building up new proteins, but Schulze could check the synthetic power of germinating plants by keeping the latter in dark rooms. In such "etiolated" plants, the proteolytic enzyme gives rise to the ordinary amino-acids just as do strong boiling acids or trypsin and erepsin. Furthermore, some fruits which resemble seeds from a biological point of view, yield powerful proteolytic enzymes, *e.g.*, the bromelin of the pineapple[3] and the papain or papayotin of the papaw plant,[4] which have often been studied. They give rise both to peptones and amino-acids, and we cannot tell with certainty whether they consist of two enzymes, *i.e.*,

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[1] W. Connstein: Ergebnisse d. Physiologie; iii., Biochemie, 1904.

[2] E. Schulze: Zeitschr. f. physiolog. Chem., 24 (1898); 26 (1899); 30 (1900); 47 (1906).

[3] R. H. Chittenden: Journ. of Physiol., 15 (1883).

[4] R. Neumeister: Zeitschr. f. Biol., 26 (1890).—L. B. Mendel, Connecticut Academy, 1901; Amer. Journ. of Medical Sciences, 1902.—F. Kutscher: Zeitschr. f. phys. Chem., 46 (1905).

a zymogen and activator like pepsin and erepsin, as has been suggested by Vines,[1] or of one enzyme, like trypsin, which splits proteins partially. Nor do we know if in plants germinating proteins are and must be completely disintegrated as in animal digestion. Proteolytic enzymes are found in flowers, leaves, and other parts of plants,[2] but they occur in smaller amount or are less powerful than the enzymes of seeds. They do not resemble the digestive enzymes, but the autolytic enzymes of tissues.

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[1] S. H. Vines: Annals of Botany, 17–20 (1903–1906).
[2] A. Kossel: Zeitschr. f. physiolog. Chem., 49 (1906).

CHAPTER XV

The Hydrolytic Enzymes of Tissues, or Autolytic Enzymes

For reasons mentioned, the knowledge of these enzymes, which are not secreted and not destined for secretion, is rather incomplete. These enzymes have been studied particularly in recent years, first because we have gradually learned better methods of obtaining them, and secondly, on account of their supposed biological and clinical interest. We know, since the labors of Voit, that the non-infectious diseases, like diabetes and gout, do not affect the general metabolism of our body. The quantity and character of the products which are finally formed in the metabolism of proteins or carbohydrates, is the same in disease as in health. Therefore, many physiologists and physicians have hoped that the intermediate metabolism intercalated between the digestion organs and the organs of elimination, could give us some explanation of the riddles of disease. In recent years much has been done regarding the enzymotic processes taking place in tissues, and the enzymes responsible for these processes.

We can separate the tissue-enzymes into three classes: first, the hydrolytic; second, the oxidizing; and third, the metabolism enzymes.

The hydrolytic enzymes of tissues are mainly the same

as the enzymes of the alimentary canal. We have no evidence of any difference in properties, solubility, or action, between the diastase in saliva or pancreatic juice, and the diastase in the liver, the muscles, or the blood; or between the lipase of the pancreatic juice and that of the liver. The only difference has been mentioned before, *i.e.*, the difficulty of extracting the intracellular enzymes, and on account of this difficulty we may be deceived by delusive differences obtained in their use.

Another difficulty is the separation of blood-enzymes from the tissue-enzymes, *i.e.*, the removal of blood from organs. Washing out blood completely is impossible *post mortem*, and is not easy during life.[1]

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[1] O. Cohnheim and D. Pletnew: Zeitschr. f. physiolog. Chem., 68 (1910).

CHAPTER XVI

Proteolytic Enzymes of Blood

Under normal conditions, blood plasma does not contain enzymes; but the blood plasma is the path for all substances from one organ to another, from the places of absorption to the places of utilization and elimination. It is possible that the enzymes of the alimentary canal, which are found in the products of their action, are absorbed together with these products. Pepsin is found in small amount in the urine,[1] and before elimination, pepsin must have passed through the blood. Weinland [2] has studied this question by means of invertin, which occurs only in the mucous membrane of the small intestine, and nowhere else in the body, and of which the smallest traces can be recognized. Blood plasma contains no invertin, but it was possible to find the invertin in the plasma after repeated subcutaneous injections of cane sugar in puppies. The experiment gives evidence that cells producing enzymes and secreting them in one direction, can change this one-sided polarity and throw the enzyme backward into the blood, when the stimulus comes to them from the opposite direction. But we learn further from the experiment that, under normal conditions, cells deliver the enzymes in only one direction and that

[1] M. Matthes: Arch. f. exper. Path. u. Phar., 40 (1904).

[2] E. Weinland: Zeitschr. f. Biol., 47 (1905).

blood-plasma contains no enzymes arising from the digestive organs. Only when the cells are destroyed, as in phosphorus poisoning, do they, and with them the enzymes, enter and circulate in the blood in great amount.[1] As blood clots, fibrin is formed, but the clots are dissolved in a short time. The livers of dogs or men killed by phosphorus soften and dissolve after a few hours.

All blood corpuscles contain enzymes. In red corpuscles[2] and in blood-platelets, proteolytic enzymes are found, which convert peptids into amino-acids like erepsin. This ferment of blood-platelets is, according to Deetjen, involved in the clotting of blood (see below). More important are the proteolytic enzymes of the white corpuscles. The white corpuscles are complete organisms, independent in many respects of the rest of the body, and living like protozoa. They eat food, take in oxygen and give out carbonic acid, dissolve and dissociate solid proteins such as fibrin, digest the bodies of micro-organisms and store up glycogen, fat, and other "granula." Leucocytes occasion the resolution of the solid exudate in the lungs of croupous pneumonia, and develop the proteolytic power of pus which softens the tissues. That white corpuscles contain a proteolytic enzyme, has been known for a long time. Recently, Opie[3] has pointed out that

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[1] M. Jacoby: Zeitschr. f. physiolog. Chem., 30 (1900).—A. J. Wakeman: *ibid.*, 44 (1905).

[2] E. Abderhalden and H. Deetjen: *ibid.*, 51 and 53 (1907).

[3] E. L. Opie: "Studies of the Rockefeller Institute," 4 (1905); 6 (1906); 8 (1908).—A. R. Dochez: 10 (1910).

two different enzymes occur in those leucocytes which act especially as phagocytes. The polynuclear leucocytes with fine granulations contain an enzyme which causes proteolytic digestion in a neutral or alkaline medium, and which is almost wholly incapable of action when placed in an acid medium. Opie gives to this enzyme the name "leucoprotease." The large mononuclear leucocytes, the macrophages of Metchnikoff, contain an enzyme which is incapable of digesting protein in an alkaline medium, but is active in the presence of a weak acid, for instance 0.2 per cent acetic acid. Stronger acid, or hydrochloric acid, checks the action. Opie suggests for this enzyme the name "lymphoprotease."

In life, both enzymes are limited to intracellular action. It is known that intracellular digestion by many unicellular organisms, amœba and others, occurs in the presence of acid. The protoplasm manufactures a little temporary stomach within its substance. Similarly, leucocytes engulf and ingest bacteria and other micro-organisms, red corpuscles, etc., and by means of their enzymes they dissolve the protein of these structures. These enzymes are not involved in the digestion, nutrition, or metabolism of the human or animal body, but in phagocytosis and immunity.

The conditions are changed after the death and mechanical or chemical disintegration of leucocytes. Then, leuco- and lymphoprotease are set free, and can either attack the proteins of tissues outside of the leucocytes, or the action is checked by the anti-enzymotic action of the serum

albumin surrounding cells (*vide supra*). Investigation of the proteolytic enzymes of leucocytes is difficult. We meet with two different enzymes having different optima and different degrees of resistance to high temperature, and we meet with a substance which can check the action. The detection of these enzymes is now facilitated by using a plate of coagulated blood according to the method of Müller and Jochmann.[1] Traces of enzyme brought on the surface of the plate are detected by the appearance of a cavity formed by solution of a portion of the solid protein of the plate.

There can be no doubt that the traces of proteolytic enzymes occasionally found in serum or in fibrin,[2] are the enzymes derived from dissolved leucocytes. The enzymes of inflammation and pus, which destroy tissues, are leuco- and lymphoprotease. Opie suggests—and I think he is right—that the enzyme observed by him in bone marrow is identical with leucoprotease, and the enzyme of lymphatic gland with lymphoprotease. From the spleen, Hedin [3] has extracted two enzymes, one acting in an acid, the other in an alkaline medium, and to which he gives the names lienoprotease α and β. The great quantity of all forms of leucocytes in the spleen suggests to us the identity of these with Opie's enzymes. Disinte-

[1] E. Müller and Jochmann: Münchener med. Woch., 1906.—Hoffmeister's Beitr., 11 (1909).

[2] B. T. Barker: Rockefeller Institute, 8 (1908).

[3] S. G. Hedin: Zeitschr. f. physiolog. Chem., 32 (1901).—C. M. Takamura: *ibid.* 63 (1909).—T. B. Leathes: Journ of Physiol., 28 (1902).

gration of protein has been observed in the thymus,[1] which is rich in leucocytes. In leukemia, the spleen and lymphatic glands may be enormously enlarged, and in such cases it has been observed that strong proteolytic enzymes split the proteins of the spleen or the glands. The increased quantity of the enzymes permits a study of their action, which is identical with that of erepsin or trypsin. Proteins are converted first into peptones, and then into amino-acids.[2]

Blood yields perhaps a small quantity of arginase.[3]

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[1] Fr. Kutscher: Zeitschr. f. physiolog. Chem., 34 (1901).
[2] O. Schumm: Hofmeister's Beiträge, 7 (1906).
[3] A. Kossel and H. D. Dakin: *ibid.*, 42 (1904).

CHAPTER XVII

Proteolytic Enzymes of Tissues

Salkowski [1] and Jacoby [2] have described an autodigestion or autolysis of liver and other organs. If we allow liver extracts to stand for several days or weeks at the temperature of the body, a part of the proteins is split and converted first into peptones, and then into amino-acids. Since the first publication of Jacoby, much work has been devoted to the study of autolysis. It was thought these observations threw light on the processes of the intermediate metabolism of proteins, because earlier observations had shown that the body and all organs lose protein in starvation and during fever, and that the tissue-protein can be drawn upon in metabolism. But Vernon [3] demonstrated that the proteolytic enzymes of the kidney, liver, and other organs resemble erepsin. They rapidly convert peptones into products not giving the biuret reaction, but they attack tissue-proteins only very slowly and incompletely. Later, Abderhalden,[4] using artificial

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[1] E. Salkowski: Zeitschr. f. klin. Med., 17 Suppl. (1891).

[2] M. Jacoby: Zeitschr. f. physiolog. Chem., 30 (1900); 33 (1901).—Hofmeister's Beitr., 3 (1903).—S. G. Hedin and S. Rowland: Zeitschr. f. physiolog. Chem., 32 (1901).

[3] H. M. Vernon: Journ. of Physiol., 32 (1904); 33 (1905).

[4] E. Abderhalden (and collaborators): Zeitschr. f. physiolog. Chem., 49, 51, 53, 55 (1906–1908).

peptids, confirmed the erepsin-like character of the tissue-enzymes. The ereptic tissue-enzymes are not identical with the blood-enzymes, because they are found in organs completely freed from blood.[1] This erepsin is most abundant in the kidney (sometimes present in larger quantity than in the intestinal mucosa), and less abundant in the liver and the spleen; still less in the lungs and brain, and skeletal muscles yield only traces.

A second tissue-enzyme is arginase,[2] already cited as a digestive enzyme of the small intestine. According to Kossel and Dakin, most arginase is found in the liver, less in the kidney, small intestine, and thymus and lymphatic glands. Spleen and muscles do not seem to yield arginase.

The function of tissue-erepsin and tissue-arginase is not certain. They either have a function in the metabolism of the liver and kidney; a view which is supported by Jacoby, and especially by Vernon, who designates the ereptic power of tissues as a measure of functional capacity (and the failure of erepsin in muscle recalls the fact that the working muscle does not burn protein, but burns carbohydrates and fats)—or, erepsin and arginase attack those peptones and arginine in the liver which have escaped the enzymes of the intestine in the course of absorption. There are some peptones, if injected subcutaneously, without the intervention of the alimentary canal, which are retained in the organism and converted into

[1] E. Bloch: Biochem. Zeitschr., 21 (1909).—O. Cohnheim and D. Pletnew: Zeitschr. f. physiolog. Chem., 68 (1910).

[2] A. Kossel and H. D. Dakin: *ibid.*, 41, 42 (1904).

urea and carbonic acid in the ordinary way.[1] The erepsin of the kidney might be high because similarly retained by that organ though destined for elimination.

Our knowledge of the proteolytic enzymes of the tissues and their function in metabolism is unsatisfactory. Some investigators have studied the enzymes of cancers[2]; they believe that malignant tumors, while rapidly increasing, push away the tissues as an abscess does, and they have tried to detect enzymes in such cancers. But the cancer grows in another way than does the softening abscess, and the differences found between normal tissue and cancer are so small that they can be easily explained by the amount of blood and the well-known inflammations in the neighborhood of tumors. It would be interesting to look for enzymes in connective tissue. When we see that in pregnancy the tissues are loosened in a remarkable way, we might suppose that proteolytic enzymes are fundamentally responsible for the process.

There are examples in the lower animals of a rapid solution of tissue under special conditions. The disappearance of the tail of the gnat or the chewing apparatus of the caterpillar are such examples. In some of these cases, Metchnikoff and his pupils have observed an energetic phagocytosis; the enzymes of leucocyte dissolves the

[1] R. Neumeister: Zeitschr. f. Biol., 24 (1888).—H. Friedenthal and M. Lewandowski: Arch. f. (Anat. u.) Physiolog., 1899. Suppl.—L. B. Mendel and E. W. Rockwood: Amer. Journ. of Physiology, 12 (1904).—F. P. Underhill: *ibid.*, 9 (1903).

[2] E. Abderhalden, Medigreceanu, and Pinkussohn: Zeitschr. f. physiolog. Chem., 66 (1910).

organs which have become needless. But in other cases no phagocytosis is seen, and the solution of the solid tissues must be effected by proteolytic ferments which are produced or activated in the tissues as they are needed. The testicles of the salmon grow in starving animals, and the protein is derived from the muscles. Protein of muscles and testicles are widely different, but according to Kossel and Weiss,[1] proteins of muscles yield enough arginine and lysine to be converted into testicle-protamine, and a proteolytic enzyme in the muscles would explain the process. Also in pregnancy, it seems that the proteins of the mother constitute the material which is used by the growing embryo, and therefore the tissues of the mother must be dissolved. But the proteolytic ferments which provoke this autolysis are not known.

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[1] A. Kossel: Zeitschr. f. physiolog. Chem., 44 (1905).—F. Weiss: *ibid.*, 52 (1907).

CHAPTER XVIII

Other Hydrolytic Enzymes of the Blood and Tissues

Leucocytes probably contain diastase, because we can see within them microscopical granules giving the color reactions of glycogen. After clotting, blood serum yields diastase [1] in small amount, probably derived from leucocytes. The tissues, and especially the liver, are much richer in diastase. In the liver-cells it lies inside of the protoplasm, and here the diastase must act. This is the reason that the extraction of diastase from the liver is difficult, and the amount of enzyme seems to be small. A real difference between the carbohydrate-splitting enzymes of the alimentary canal and those of the tissues is, that in the tissues, diastase and maltase always occur in the same place, and cannot be separated. Glycogen is thus converted into glucose. Diastase and maltase are found in many or all organs, which is in harmony with the general occurrence of glycogen. They are found in great amount in the liver, in muscles, and in the placenta. As to the diastase of the liver, Bang [2] has reported that the quantity found in pieces of the organ depends upon the behavior

[1] C. Hamburger: Pflüger's Arch., 60 (1895).—E. Fischer and Niebel: Berliner Akad. d. Wissenschaften, 1896.—W. Kühne: Heidelberger Naturhist.-med. Verein, N. F. (1877).

[2] I. Bang: Hofmeister's Beitr., 10 (1908).

of the animal immediately before death, and that it can be changed by nervous stimulation, by the activity of the liver, etc. Other carbohydrate-splitting enzymes in the body are not known.

Whether blood and blood corpuscles contain lipase in traces or in great amount, is questioned.[1] In the tissues, lipase is found in large quantity in the liver. It is identical with the pancreatic lipase, or resembles it closely, because it is activated by the bile, and acts best in a neutral medium (*vide supra*). According to Nencki,[2] muscle and kidney contain weak lipases; the other tissues, especially the adipose tissue, have not been investigated.

Some tissues contain nuclease, the same enzyme which occurs in the pancreas and the small intestine, but it is not determined whether the enzyme originates in the cells of the tissues, or comes from the leucocytes. It is found in greatest amount in the spleen [3] and the thymus,[4] organs rich in leucocytes.

That tissues contain lecithases is not astonishing, because the steapsins dissociate lecithin; thus choline is found among the products of autolysis of the liver and other organs. According to Sieber, lipase of the blood-serum does not attack lecithin.

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[1] C. Schumow-Simanowski and N. Sieber: Zeitschr. f. physiolog. Chem., 49 (1906).—W. Connstein: Ergebnisse d. Physiologie, 333, Biochemie (1904).

[2] (M. Nencki and) E. Lüdy: Arch. f. exper. Path. u. Pharm., 25 (1889).

[3] O. Schumm: Hofmeister's Beitr., 7 (1906).

[4] Fr. Kutscher: Zeitschr. f. physiolog. Chem., 34 (1901).

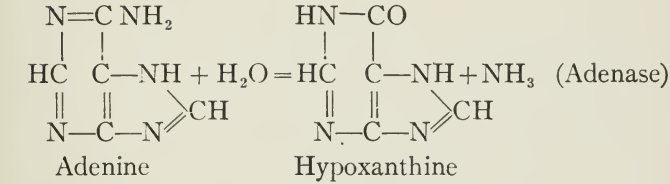
CHAPTER XIX

Urease and Nucleases

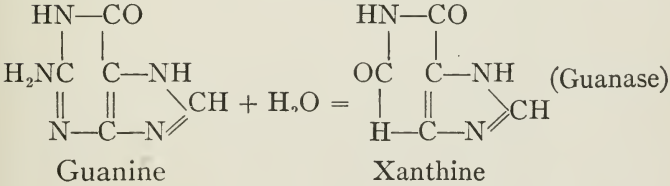
These enzymes break the connection between nitrogen and carbon with the entrance of water, as do the hydrolytic enzymes, but differ greatly from the proper hydrolytic enzymes. They bring about the following reactions:

$$H_2NCNH_2 + 2H_2O = CO_3H_2 + 2NH_3 \quad \text{(Urease)}$$

Urea — Carbonic Acid — Ammonia



Adenine — Hypoxanthine

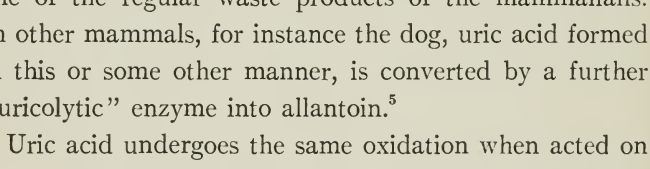


Guanine — Xanthine

Urease is found in many bacteria which provoke fermentation of urine eliminated from the body, or, in pathological cases, within the bladder. It has not been observed in the organs of the animal body. According to Miquel, we can extract and separate urease from the

bodies of the bacteria.[1] Adenase and guanase were first found in yeast by Lehmann [2] in Kossel's laboratory. In recent years they have been found in the liver, kidney, and other organs and investigated by Schittenhelm [3] and Jones [4] and their collaborators. They can be easily dissolved. According to Schittenhelm, xanthine is converted by this enzyme in the presence of abundant oxygen into uric acid, showing that under these conditions the enzyme provoked an oxidation instead of an hydrolysis. Thus the purin-bases, adenine and guanine, cleavage-products of nucleic acid split by nucleases, are converted into the oxypurins, and possibly they may be oxidized further into uric acid, one of the regular waste products of the mammalians. In other mammals, for instance the dog, uric acid formed in this or some other manner, is converted by a further "uricolytic" enzyme into allantoin.[5]

Uric acid undergoes the same oxidation when acted on by a mild oxidizing agent, such as potassium permangan-



Uric acid Allantoin

[1] Miquel: Compt. rend., 111 (1890).

[2] V. Lehmann: Zeitschr. f. physiolog. Chem., 9 (1885); cf. K. Shiga, *ibid.*, 42 (1904).

[3] A. Schittenhelm: *ibid.*, 42, 43 (1904); 45, 46 (1905); 48, 50 (1906).

[4] W. Jones: *ibid.*, 41, 42, 44, 48.

[5] W. Wiechowski: Hofmeister's Beitr., 9 (1906).

ate. In other animals, like man, allantoin is formed and eliminated, but nevertheless a uricolytic enzyme capable of destroying uric acid has been extracted from the organs of man, the ox, and pigs, by Wiechowski, Jones, and Schittenhelm. It is not known how this enzyme acts; whether there is a stronger oxidation or no oxidation.

It seems that similar aminolytic, or desaminating, enzymes, which replace the NH_2 group by the OH group, are widely distributed in organs. The specificity of these enzymes is not very evident. Schittenhelm supposes that only one enzyme attacks adenine and guanine; Jones suggests the existence of two or three enzymes, and it is not impossible that the same enzyme which attacks, for instance, alanine, and converts it into lactic acid, attacks likewise adenine and guanine. If this is so, we must be very slow to draw conclusions regarding specificity, because we cannot know that an enzyme studied is destined for the special reaction which we employ it to provoke.

CHAPTER XX

The Oxidases

Food is burned in and by the living protoplasm, and thus supplies the energy needed for life. This combustion could be an anoxybiotic process, but, in the higher animals, combustion, which takes place continuously in the tissues, is an oxidation, and attempts have for a long time been made to detect and extract enzymes which oxidize substances, especially sugar or fat. Some enzymes have been found which convert aldehydes into acids, or produce similar reactions, and act only in the presence of oxygen. Other substances have been discovered, which liberate oxygen from hydrogen peroxide, and the oxygen thus set free can produce oxidative reactions. Whether in these last substances we are dealing with enzymes or with agencies of a wholly different type, is not certain. The facts and observations described by the different authors have not been generally accepted. Though the study of such enzymes is of great interest, a good deal of the experimental work has been very inexact. It is important that we separate the main metabolism-enzyme of the type of zymase from the so-called oxidases. The latter group of substances can now be briefly reviewed.

1. Catalase. Schönbein found in 1863, that hydrogen peroxide is decomposed into oxygen and water by platinum black and other inorganic substances, and likewise by

blood, milk, tissues, etc. In the last few years, Bredig and his pupils have studied the decomposition of hydrogen peroxide by organic and inorganic catalysts, and have tried to support Schönbein's view, that this decomposition by colloidal platinum, gold, or mercury, resembles enzyme action. Many finely divided substances, especially the colloidal solutions of the metals platinum, gold, and mercury, produce this decomposition. Tissues and animal fluids are also colloidal solutions, and provoke the same reaction. If the colloidal properties of the tissues or the blood are destroyed by heating, they lose the capacity of decomposing hydrogen peroxide. Because enzymes are destroyed by heating, there seems to be a resemblance between inorganic catalysts and enzymes. But no further proof has been brought forward that the two processes are of a similar character. Hydrogen peroxide never occurs in our bodies, and I do not believe that the "catalase" is an enzyme.[1]

2. The following reactions are more or less well defined and cleared up, and there can be no doubt that they are of enzymotic nature. The oxidases oxidize substances readily oxidizable in the presence of atmospheric oxygen.

a. ALDEHYDASE.[2] This converts salicylaldehyde into salicylic acid:

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[1] G. Bredig: Ergebnisse d. Physiologie, I. Biochemie (1902); M. Jacoby: *ibid.*, I. Biochemie (1902).—A. Bach and R. Chodat: Biochemisches Zentralblatt, i. (1903); viii. (1909).—Raudnitz: Zentralbl. f. Physiologie, 12 (1899).—Spitzer: Pflüger's Arch., 67 (1897).

[2] M. Jacoby: Ergebnisse d. Physiologie, I. Biochemie (1902).

$$C_6H_4\begin{cases}OH\ (ortho)\\CHO\end{cases} + O = C_6H_4\begin{cases}OH\ (ortho)\\COOH\end{cases}$$

or benzylaldehyde into benzoic acid: $C_6H_5CHO + O = C_6H_5COOH$: or, formaldehyde into formic acid; $HCHO + O = HCOOH$, and other similar reactions. Salicylic acid can be detected and estimated easily by the red color reaction with ferric chloride, and after adding salicylaldehyde, the red color occurs in many tissues, especially in the liver, spleen, kidney, and suprarenal glands. Blood gives the reaction to a slight extent. Jaquet,[1] Abelous and Biarnés[2] and Salkowski,[3] have demonstrated that the oxidation of salicylaldehyde is effected by an enzyme, which can be extracted from the tissues, and Jacoby[4] has freed the oxidizing enzyme from proteins and most other impurities. The aldehydase of liver studied by him, is an enzyme, the solubility and precipitability of which are best known, and my description of the chemical properties of enzymes is based chiefly on Jacoby's work on aldehydase. He obtained a clear, aqueous solution, which could convert salicylaldehyde into salicylic acid in a short time and in great amounts. The aldehydase is very resistant to heat and other influences,

[1] Jacquet: Arch. f. exper. Path. u. Pharmakol., 29 (1892).

[2] Abelous and Biarnés: Arch. de Physiol. et de Path. gén., 1894, 1898.

[3] E. Salkowski: Virchow's Arch., 147 (1898); Zeitschr. f. physiol. Chem., 13 (1889).

[4] M. Jacoby: Zeitschr. f. physiolog. Chem., 30 (1900).—A. Medwedew: Pflüger's Arch., 81 (1900).

and is destroyed only at 80° C. It is resistant to alkalies, but acids destroy it. It is possible, but not quite certain, that formaldehyde and salicylaldehyde are oxidized by the same enzyme. As Jacoby[1] has pointed out, the embryos of young pigs contain no aldehydase, while the adults do.

b. Laccase. This has been detected and especially studied by the French physiologist, Bertrand.[2] It converts hydroquinone into quinone:

$$2C_6H_4\begin{matrix}OH\\OH\end{matrix} + O_2 = 2C_6H_4\begin{matrix}O\\|\\O\end{matrix} + 2H_2O.$$

It converts pyrogallic acid, with a simultaneous combination of three molecules into one, forming furfurogallin:

$$3C_6H_3(OH)_3 + O_2 = HOC_6H_3\begin{matrix}O.OC_6H_3(OH)_2\\O.OC_6H_3(OH)_2\end{matrix} + 2H_2O$$

It likewise oxidizes and condenses gallic acid and other phenols into the corresponding quinones.

The compounds formed by laccase are brown or black, and the action of the laccase can be recognized by the rapid change of color into brown or black in the presence of air. Among these reactions is the oxidation of urushic acid or laccol into oxyurushic acid:

$$C_{14}H_{18}O_2 = C_{14}H_{18}O_3.$$

[1] M. Jacoby: Zeitschr. f. physiolog. Chem., 33 (1901).

[2] G. Bertrand: Compt. rend., 118, 120, 123, 124; Compt. rend. de la Soc. de Biol., 121 (1897–1898).—B. Slowtzoff: Zeitschr. f. physiolog. Chem., 31 (1900).

This reaction was the first fermentative oxidation to be investigated, and led to the discovery of laccase. The sap of the East Asian lac tree contains laccase and urushic acid, or laccol, and the laccase converts urushic acid into oxyurushic acid, as was observed by Yoshida in 1893 and explained by Bertrand in 1894. The product of the conversion is the insoluble black oxyurushic acid, which thus gives rise to the brilliant black lustre of the lacquer manufactured in Japan and China. But the laccase has been found also in many plants, such as the roots of the beet, carrot, and turnip; in the potato, apple, and pear, in clover and asparagus, in germinating seeds, in some flowers, and in many fungi. Laccase is associated with manganese, which is its activator or co-ferment (*vide supra*).

c. Tyrosinase.[1] This enzyme resembles laccase in action and occurrence, but is not associated with manganese. It converts phenols, like xylenol, amido-phenols, indophenol, phenolphtalein, naphthol, and similar compounds, into substances richer in oxygen. In most cases, these substances are at the same time combined to form larger molecules. For instance, amidophenol and phenol are oxidized and combined to form indophenol.

$$C_6H_5OH + C_6H_4(NH_2)(OH) + O = HN\begin{array}{l}C_6H_4-O\\ \quad\quad\quad\quad\ |\\ C_6H_4-O\end{array} + H_2O.$$

Indophenol has a blue color; the other products formed

[1] H. Steudel: Deutsche med. Woch., 1900.—M. Gonnermann: Pflüger's Arch., 89 (1902).

by the tyrosinase are brown or black. The enzyme has received its name because it seems to act upon the cleavage product of proteins, tyrosine, which is a phenol. If we add a tyrosinase solution to tyrosine, it becomes first red, later black, and then forms a black precipitate. But according to E. Schulze,[1] extracts of plants, which are acted upon by tyrosinase, do not always contain tyrosine. It is possible, therefore, that it is not tyrosine that is oxidized, but an impurity which often accompanies the tyrosine obtained from proteins. Until the relations are completely cleared up, however, it will be better not to change the name of the enzyme.

Tyrosinase occurs, like laccase, in many plants, especially in fungi, for instance, in Russula and Argaricus, which are the best objects for demonstrating oxidases. In animals it occurs in the blood or in the hemolymph of certain butterflies and other insects, in the intestinal juice of the meal worm, in the larva of Tenebrio molites, in the crayfish, and other higher and lower animals. v. Fürth and Schneider[2] have observed that the black precipitate formed by tyrosinase resembles in properties, cleavage-products, and in composition, the melanins naturally occurring in the skin, hair, and other organs, and in the ink-bag of the Sepia; and v. Fürth has also shown that the ink-gland and the ink-bag of the Sepia contain much tyrosinase, which might perhaps explain the formation of the ink-melanin in the Sepia. The three oxidases also

[1] E. Schulze: Zeitschr. f. physiolog. Chem., 50 (1906).

[2] O. v. Fürth and H. Schneider: Hofmeister's Beitr., 1 (1901).

convert guaiaconic acid, $C_{20}H_{24}O_5$, into guaiacum blue, $C_{20}H_{22}O_6$. The guaiac test, the blue color with guaiac tincture or the pure acid, is a very easy and convenient test for oxidases, and is often used by investigators.

The differences between the individual oxidases are not well understood. Enzymes associated with manganese must be of an individual type. And I think we are right in separating the aldehydases converting aldehydes into acids, from the tyrosinase, which on the contrary forms aldehyde-like compounds. It must be mentioned that salicylaldehyde, pyrogallol, etc., do not occur in the organisms; in using them we are not working with the natural substrate for the oxidases, and the evidence that an individual extract attacks only aldehydes, or only hydroquinone, or only gives the guaiacum-blue test, is not very conclusive. The question as to the number of oxidases remains open.

The compounds which are acted upon by the three oxidases are readily oxidizable in an alkaline medium, and absorb oxygen even without oxidases. Hydroquinone, pyrogallol, and amidophenol are used in photography as reducing substances; and pyrogallol is also employed in gas analysis because of its oxygen-absorbing properties. The oxidases seem only to accelerate the oxidation, and enable it to take place in a weakly alkaline medium; while without enzymes, oxidation requires more alkali and more time. The oxidases are oxygen bearers or carriers, and the view regarding enzymes as mere catalysts is based upon the properties of these oxidases.

The presence of oxygen is necessary for the action of oxidases, and reactions are best observed with a current of air passing through the reaction-flasks. For the action of laccase associated with manganese, Bertrand suggests a scheme to represent the carrying of oxygen in a water-solution from the air to the substrate of the enzyme.

$$R''Mn + H_2O = R''H_2 + MnO.$$
$$MnO + O_2 = MnO_2 + O.$$
$$R''H_2 + MnO_2 = R''Mn + H_2O + O.$$

Thus after the reaction, the solution contains the enzyme R″ in an unchanged state, and O not as an inactive molecule, but as free uncombined oxygen in a nascent state. The action of the laccase, according to this theory, would be to convert the inactive oxygen of the air into the more energetic form. Aldehydase and tyrosinase do not contain manganese, which is never found in the body of higher animals. Spitzer has observed that, in solubility, the oxidases agree with nucleoproteins. In some nucleoproteins, iron was observed, and Spitzer has supposed that in these oxidases manganese is replaced by iron. The supposition is rejected by Sauerland [1] and Masing,[2] who failed to find any iron in nucleic acid and in the eggs of the sea-urchin, which undergo a strong oxidation during development. Jacoby [3] separated aldehydase from nucleoproteids without loss of oxidizing power. Nevertheless,

[1] F. Sauerland: Zeitschr. f. physiolog. Chem., 64 (1909).
[2] E. Masing: *ibid.*, 66 (1910).
[3] M. Jacoby: *ibid.*, 30 (1900); 33 (1901).

it seems that there is some association between oxidases and nucleoproteins, for we have seen that the hydrolytic enzymes, like pepsin, are not nucleoproteins, but in organisms are always associated with them. Warburg and Morawski [1] have observed that the absorption of oxygen by red corpuscles is closely connected with the occurrence of nucleic acid in the corpuscles, though the nucleus as a visible structure is not present.

Another scheme of reaction for oxidases and for oxidations in protoplasm, was given years ago by Hoppe-Seyler [2] and Bunge,[3] and in recent years by Bach and Chodat,[4] Kastle and Loevenhart,[5] and Dakin.[6] These investigators have supposed that, in tissues, readily oxidizable substances are formed, which have a special capacity for absorbing oxygen. By these substances the oxygen-molecule, O_2, is dissociated into two atoms; one atom is absorbed, and the other, in the nascent state, can attack other less oxidizable compounds. Hoppe-Seyler and Bunge did not discuss the chemical nature of the readily oxidizable substances; Bunge mentioned only the fact that cuprous and ferrous salts have this property, which he assumed for the tissues.

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[1] O. Warburg: Zeitschr. f. physiolog. Chem., 59 (1909).—P. Morawski: Arch. f. exper. Path. u. Pharmak., 60 (1909).

[2] F. Hoppe-Seyler: Zeitschr. f. physiolog. Chem., 2 (1878); 10 (1886).

[3] G. Bunge: "Physiologische Chemie." Leipzig, 1901.

[4] A. Bach and R. Chodat: Biochem. Zentralbl., 1 (1903); 8 (1909).

[5] A. S. Loevenhart and J. H. Kastle: Amer. Chem. Journal, 29 (1903). A. S. Loevenhart: Amer. Journ. of Physiology, 13 (1905).

[6] H. D. Dakin: Journ. of Biolog. Chemistry, 4 (1908)

Bach and Chodat, Kastle and Loevenhart, and Dakin, suppose the intermediate formation of peroxides not known in the time of Hoppe-Seyler. Bach and Chodat have described, besides the oxidases mentioned, another type, the peroxidases, which give the guaiac-blue test only in the presence of hydrogen peroxide or sodium peroxide. Experimental proofs for these latter compounds are not very conclusive, and I think that the whole theory of oxidation by reduction and reoxidation of substances which, in this way, act as oxygen carriers is not a satisfactory explanation of the process as it takes place in the body.

It is true that in addition to the oxidation of foods, there are reducing processes in the organism such, *e.g.*, as the conversion of atoxyl into arsenophenylglycine, recently demonstrated by Ehrlich [1]; or the reduction of methylene blue in the tissues immediately after death, which was used by Ehrlich [2] as a measure of the avidity of tissues for oxygen; or the conversion of acetoacetic acid into oxybutyric acid,

$$CH_3.CO.CH_2COOH \rightleftarrows CH_3CHOH.CH_2.COOH,$$

found by Blum,[3] Friedmann and Maase,[4] Dakin,[5] and

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[1] P. Ehrlich: Deutsche dermatolog. Ges., 1908.—W. Roth: Berliner klin. Woch., 1909, No. 11.

[2] P. Ehrlich: "Sauerstoffbedürfniss des Organismus," Berlin, 1890.

[3] L. Blum: Münch. med. Woch., 1910.

[4] E. Friedmann and C. Maase: Biochem. Zeitschr., 26, 1910.

[5] H. D. Dakin: Journ. Amer. Med. Assoc., 1910; Münch. med. Woch., 1910.

Neubauer.[1] But against the generalization of these processes we have three arguments.

1. Peroxides are never found in the organism, and substances which could serve as oxygen carriers occur in too small amount to be of importance. According to this theory, we are not dealing with enzymes, which can convert an enormous quantity of matter; but one equivalent weight of the reducing substance can liberate oxygen only for one equivalent weight of the less oxidizable substance, and there are also difficulties from the point of view of energetics.

2. Jacoby has purified aldehydase by a prolonged treatment. If aldehydase were a compound readily oxidizable, it must have undergone oxidation during these manipulations. He observed, also, that the pure solution does not convert salicylaldehyde according to equivalent weights. He allowed the process to go to completion and then removed the salicylic acid formed by dialysis, and added new salicylaldehyde; a new formation of salicylic acid could now be observed. This is an enzymotic process.

3. All the theories of oxidation by intermediate reduction are upset by the discovery of zymase and the enzymes resembling it which form lactic or acetic acid. These enzymes are found in expressed juices, which do not contain reducing substances, and which do not oxidize other compounds. They convert in a specific manner only those compounds which furnish energy for the vital processes

[1] O. Neubauer: Zeitschr. f. physiolog. Chem., 70 (1910).

of the individual micro-organism. They act like true enzymes, that is to say, they convert, not the equivalent weight, but an unlimited quantity.

Combustion in the protoplasm of cells or tissues is therefore occasioned by enzymes, and not by another process, such as the formation of peroxides, etc. But the combustion of sugar by zymase, the only isolated and well-investigated enzyme, is not an oxidation. It follows the equation:

$$C_6H_{12}O_6 = 2CO_2 + 2C_2H_5OH.$$

Glucose is broken down into alcohol and carbon dioxide without loss or addition of water, or addition of oxygen. Likewise, the formation of lactic acid is not an oxidation, but only a cleavage of the molecule of sugar:

$$C_6H_{12}O_6 = 2C_3H_6O_3.$$

Some investigators, for instance Bunge, have thought that such an intramolecular change may occur generally in the tissues and give rise to compounds readily oxidizable. Bunge [1] and Weinland [2] have observed that the metabolism of the round worm is a mere intramolecular change without oxidation; glucose is converted into valeric acid and carbon dioxide. Also, the pupa of the fly has, according to Weinland,[3] for a certain period of metamorphosis, an anoxybiotic metabolism. It seems possible that perhaps, in higher animals, the readily oxidizable substances are

[1] G. Bunge: Zeitschr. f. physiolog. Chem., 14 (1890).
[2] E. Weinland: Zeitschr. f. Biologie, 42 (1901).
[3] *Ibid.*, 48 (1905).

formed in the tissues, and oxidized later in the blood or lungs. That organs and the expressed juice or pulp of organs absorb oxygen, is no evidence against this theory, because we cannot always separate organs and blood, and because the final oxidation could occur in the lymph, and not in the blood-vessels. But the whole theory that the food-stuffs utilized in the tissues are chiefly split and oxidized only in a secondary process, is impossible from the energetical point of view. The investigation of the so-called respiratory quotient, the relation between the absorbed oxygen and the eliminated carbon dioxide, and the calorimetric investigations of Rubner [1] and of Atwater and Benedict,[2] have shown that carbohydrates and fats are as completely burned in the organism as in a calorimeter bomb, and that the substances deliver the same quantity of heat as in artificial combustion. It has been shown by Barcroft [3] and his collaborators, by the author,[4] and by Vernon,[5] that the respiratory quotient of the individual tissues is similar to or the same as the respiratory quotient of the whole organism.

It was shown by Frank,[6] by Zuntz,[7] and by Atwater and

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[1] M. Rubner: Zeitschr. f. Biolog., 21 (1885); 30 (1892); 42 (1901).

[2] W. O. Atwater and F. G. Benedict: Carnegie Institution, 1905.—F. G. Benedict: Amer. Journ. of Physiology, 24 (1909).

[3] J. Barcroft: Journ. of Physiology, 31, 32, 33 (1904–1907).

[4] O. Cohnheim and D. Pletnew: Zeitschr. f. physiolog. Chem., 69 (1910).

[5] H. M. Vernon: Journ. of Physiology, 35, 36, 39 (1906–1909).

[6] O. Frank: Ergebnisse d. Physiologie, 3 (Biophysik), 1904.

[7] N. Zuntz: Pflüger's Arch., 63 (1891); 68 (1897).—A. Dürig: Pflüger's Arch., 113 (1906).

Benedict,[1] that a third, or thirty per cent, of the heat produced in muscle, is converted into mechanical energy or work. Frank has directly measured the output of heat in isolated frog's muscles, using a delicate thermo-electrical method. The weight raised by the muscles gives the mechanical energy. In the best experiments this was one-half of the heat produced at the same time, so that a third of the energy produced became work, and two-thirds became heat.

Atwater and Benedict have determined the heat given off from the body in the respiration calorimeter. The external work was measured by a specially devised ergometer, and they found that a fifth of the total energy becomes muscular work.

Zuntz and his collaborators have measured the absorption of oxygen during a climb, and with the heat produced by this oxygen, they compared the weight of the man with the height reached in a given time. They found that thirty per cent of the total energy liberated in the body during muscular work becomes mechanical energy. Since the heart, the respiratory muscles, and other muscles not directly moving the body absorb an increased amount of oxygen in climbing, more than thirty per cent of the whole energy must be converted into movement or work. In the best steam engines constructed by man, a much smaller amount of the total potential energy of the fuel is utilized as work, not more than ten to thirteen per cent, the rest being lost as heat.

[1] *Loc. cit.*

If the quantitative relation of work to heat is so much better in the muscle engine than in the steam engine, we must conclude that in muscle the latent potential energy must be completely or to a very great extent set free during combustion. We know that there are conversions of sugar without oxidation or with a partial oxidation, as exemplified in the conversion into ethyl alcohol and carbon dioxide, or into lactic acid, or the conversion into glycerinaldehyde or methylglyoxal, or into acetone or ethyl- or formaldehyde. Furthermore we observe the probable conversion of fatty acids into oxybutyric acid and acetone. But all these conversions liberate only a small amount of the energy which is set free by the complete combustion of sugar or fat into water and carbon dioxide. The following table shows in large calories the heat produced by the complete combustion of 100 gm. of glucose into water and carbon dioxide, and by the partial conversion of the sugar into ethyl alcohol, lactic acid, formic acid, glyoxylic acid, glycolic acid, formaldehyde, glycerinaldehyde, glyoxal and methylglyoxal. The figures were obtained by subtracting the heat of combustion of these compounds from the total heat of combustion of the sugar. The table also gives the corresponding figures for stearic acid and three compounds which are probably derived from the fatty acids. It is hardly possible to give exact figures in all cases, because those found by the different authors vary to a certain degree, and because, for some of the compounds, the heats of combustion have not been measured. It was necessary to calculate them from the capacity of

oxygen, and it is possible that the true values are smaller or greater by 10 or 20 calories. Nevertheless, the conclusion cannot be denied that the conversion of glucose or stearic acid into one of these compounds sets free a quantity of heat which is not sufficient to explain the heat really produced in muscle by combustion of sugar or fat.

Heat of combustion of 100 gm. glucose and the corresponding quantities of

Glucose	380	Cal.	380	Cal.	=	100	per cent.
Formic acid	317	"	63	"	"	17	"
Glycolic acid	271	"	109	"	"	29	"
Glyoxylic acid	213	"	167	"	"	44	"
Lactic acid	329	"	51	"	"	13	"
Ethyl alcohol	357	"	23	"	"	6	"
Formaldehyde	362	"	18	"	"	5	"
Glycerinaldehyde	355	"	25	"	"	7	"
Glyoxal	297	"	83	"	"	22	"
Methylglyoxal	338	"	42	"	"	11	"

Heat of combustion of 100 gm. stearic acid and the corresponding quantities of

Stearic acid	975	Cal.	975	Cal.	=	100	per cent.
Acetaldehyde	877	"	98	"	"	10	"
Oxybutyric acid	745	"	230	"	"	24	"
Acetone	660	"	315	"	"	32	"

If thirty to forty per cent of the energy developed in the muscle becomes work, all conversions setting free less than thirty or forty per cent are excluded from consideration in seeking an explanation of the source of energy in muscle, for the muscle derives no energy from substances which are subsequently oxidized elsewhere, as in

the blood or lungs. Since the energy is needed in the muscles, the muscle-engine naturally cannot use energy set free elsewhere.

Less than thirty or forty per cent of the whole potential energy is set free by all the partial combustions, as shown above, except in the case of acetone arising from fat, and the glyoxylic acid, which might be formed by partial combustion of sugar. It is remarkable that of all the compounds suggested as intermediate products of combustion, only these two are found under special conditions in the urine or in the body. Nevertheless I think that it is very improbable that these or similar products are the end-products of the metabolism in muscle, and are completely oxidized elsewhere. For in that case, all energy set free in muscle must become mechanical work, and the muscle would have a ratio of efficiency of almost one hundred per cent. Because this result is very improbable, we must conclude that the source of energy for the striped skeletal muscle must be a complete oxidation of sugar or fat. The large amount of oxygen absorbed in glands, according to Barcroft, and the uniformity of the respiratory quotient in glands and muscles, supports this idea for the glands as well.

The conclusion does not hold good, perhaps, for unstriped or smooth muscles. I have measured the output of the carbon dioxide of the muscles of the small intestine in a state of activity, and Kehrer [1] did likewise with the

[1] E. Kehrer: Arch. f. Gynäkologie, 89 (1909).

smooth muscles of the uterus. The production of carbon dioxide per 100 gm. per hour was for

Striped muscle	Smooth muscles of intestine	Smooth muscles of uterus	Kidney
400 mgm.	79–90 mgm.	25 mgm.	1200 mgm.

I have determined [1] further the output of carbon dioxide in animals with striped muscles, such as insects, and in animals with unstriped muscles, such as snails and earth-worms. The production per 100 gm. per hour was for

Insects	Snails	Worms
133–295 mgm.	11–16 mgm.	8–15 mgm.

Magnus [2] has observed that the heart beats continued for thirty minutes without any oxygen, on allowing hydrogen to pass through the vessels of the heart. The absorption of oxygen has been measured in the heart according to Langendorff's method by Barcroft, who found it to be low. It seems to be demonstrated that even the central nervous system of frogs can live with but a very small amount of oxygen, if the poisonous products formed in metabolism are eliminated in time. The difference between striped muscles and glands on the one hand and the smooth muscles, the heart, and possibly the nervous system, on the other, is so striking, that the two are perhaps governed by different laws of metabolism. Heart, smooth muscles, and the nervous system are the oldest

[1] Zeitschr. f. physiol. Chem., 76 (1912).

[2] R. Magnus: Arch. f. exper. Path. u. Pharmak., 47 (1902).

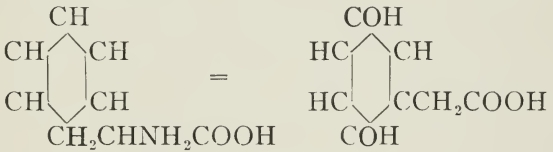
organs in phylogeny, and are of the first importance. It may be that cold-blooded invertebrates and hibernating mammals are enabled to live in starvation because of the small amount of oxygen needed for these organs. Animals with smooth muscles, like worms, or animals without movement, like the pupa of insects, or micro-organisms like yeast or the Bacillus acidi lactici, can live with an expenditure of only a small amount of energy. You see that worms, yeast, and some bacilli do indeed convert sugar without oxidation. They can live with an efficiency of the body-engine of from six to thirteen per cent.

But of the body of the higher animals more than ninety per cent of the living material consists of striped muscles and glands, and their metabolism is an oxybiotic one. The enzymes which liberate energy in the tissues of the higher animals must be oxidases.

After this digression we may now return to our main subject.

It is only a question as to whether the oxidases mentioned before—aldehydase, tyrosinase, and laccase—are the true metabolism-enzymes, or whether they have other functions. The tyrosinase and the laccase convert the aromatic cleavage products of the proteins into compounds which grow gradually darker, and become immediately insoluble in the fluids of the animal or vegetable organism. There can be no doubt that the substances thus formed are not used further in metabolism. In the case of the lac tree or the hemolymph of insects, they have, perhaps, a protective value. The tyrosinase in the green leaves of

trees produces the red, yellow, or brown colors which are so ravishing during the Indian summer, but they attack only the proteins of the dead or dying cells. The tyrosinase in the ink-bag of the sepia, and certainly all other oxidases, have an important, special function, but do not set energy free for utilization in the processes of life. Whether tyrosinases occur in the organism of higher animals, is not determined. In human pathology, cases of the so-called alkaptonuria are known. In such cases, all the tyrosine and phenylalanine of the food is converted into homogentisic acid:



This resembles the conversion of tyrosine by tyrosinase, because the substances formed darken in the presence of the oxygen of the air. The urine of patients with alkaptonuria darkens in a few minutes or hours, and thus the abnormality is detected. It is not certain whether homogentisic acid is normally formed in the metabolism of all men, and the further conversion of the homogentisic acid checked by another enzyme or group of enzymes, or whether the normal organism burns tyrosine in some other way. In the first case, we would have to conclude that the human body contains an enzyme resembling tyrosinase.

The aldehydase of the liver and other organs may be an enzyme destined for use in metabolism. It attacks

not only salicylaldehyde, but other aldehydes as well. The sugars are aldehydes, and Neubauer [1] has demonstrated that phenylalanine, and perhaps other amino-acids, become first keto- or aldehydo-acids. Thus the action of aldehydase is perhaps the first step in the definite combustion of the food. Its own action sets free no energy, or but a very small quantity, but it prepares the sugars, etc., for further conversion. It is possible that aldehydase has special functions, not known to-day, and that we may have to separate the oxidases thus far mentioned from the metabolism-enzymes in a strict sense; like zymase and the glycolytic enzyme of muscles.

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[1] O. Neubauer: Deutsch. Arch. f. klin. Med., 95 (1909).

CHAPTER XXI

The Metabolism-Enzymes

The metabolism-enzymes in the strict sense bring about the combustion of food, and set free the energy needful for life. They are the most important of all enzymes, because digestion and all other conversions are only preparatory for the real combustion of food. But the investigation of these enzymes is more difficult than is the investigation of the enzymes already cited.

That combustion in protoplasm is the work of enzymes has been for a long time assumed by many investigators. But more recent students have held that no chemical substance can oxidize sugar or fat or protein, but that the protoplasm-engine with its peculiar structure can alone carry out this process. The question was answered by Buchner's discovery of zymase, which causes the conversion of sugar into carbon dioxide and alcohol, and which delivers all the required energy for the life of the yeast. Buchner [1] was able to extract from two other micro-organisms similar enzymes, which convert sugar into lactic and acetic acid. This discovery justified the expectation that corresponding enzymes would be detected in the organs and tissues of animals.

[1] E. Buchner and collaborators: Ber. d. deutsch. chem. Ges., 1896–1908.—E. and D. Buchner and M. Hahn: "Die Zymasegärung," Munich, 1903.

The first of such metabolism-enzymes found in the tissues of higher animals is one[1] yielded by the muscles of dogs, cats, and oxen. This enzyme attacks glucose and changes it so that Fehling's or Pavy's or Trommer's test no longer shows the presence of sugar. The exact chemical process has not as yet been determined, and we have no decisive proof that we are dealing with the true combustion of glucose, which, as already mentioned, must in muscles be complete in order to furnish the requisite amount of energy. We meet here with a complication not occurring in the unicellular organisms. Two substances, from two different organs, must unite to form the enzyme converting the sugar; one substance originating in the muscles themselves, the other produced in the pancreas, and reaching the muscles only when needed.

Further evidence for the enzymotic nature of combustion in metazoa and higher animals has been brought forward by recent observations of Warburg.[2] J. Loeb has suggested, and Warburg has supported his view, that oxidation in cells is dependent on the presence of the nucleus. And Warburg has observed young, red corpuscles in human and rabbit's blood, which show a metabolism, absorb oxygen, and give out carbon dioxide, but possess no visible nucleus. They are newly formed in the red marrow after the loss of large quantities of blood, but they have already lost the nucleus, and cannot be

[1] O. Cohnheim: Zeitschr. f. physiolog. Chem., 30, 42, and 47 (1903–1906).

[2] O. Warburg: Zeitschr. f. physiolog. Chem., 57, 60 (1909).

colored with alkaline stains which show affinity for the nucleus. Nevertheless these corpuscles yield nucleic acid, and Warburg [1] and Morawitz [2] were able to observe relations between the quantity of nucleic acid and the amount of oxygen absorption. In this case the structure of the nucleus is lost, but the absorption of oxygen continues, and must, therefore, be provoked, not by any structural engine, but by a chemical substance, that is to say, by an enzyme.

Further evidence, though not so conclusive, is given by the experiments of Vernon,[3] in which absorption of oxygen and production of carbon dioxide continued for a long time, when he placed surviving kidneys and other organs in well-oxygenated Ringer's solution. It appears that cells perish early and suddenly, and at the moment of dying the amount of gaseous metabolism diminishes, but continues to a certain extent, and, even during this time, the tissues absorb oxygen and yield carbonic acid in the same ratio as before. According to Vernon, this signifies that real respiratory substances exist which occasion combustion under these as well as normal conditions.

All the foregoing considerations lead to the expectation that the metabolism of higher animals will be proved to be due to enzymes, and that only the technical difficulties of investigation have thus far prevented us from discover-

[1] O. Warburg: Zeitschr. f. physiolog. Chem., 59 (1909).
[2] P. Morawitz: Arch. f. exper. Path. u. Pharm., 60 (1909).
[3] H. M. Vernon: Journ. of Physiology, 35, 36, and 39 (1906–1909).

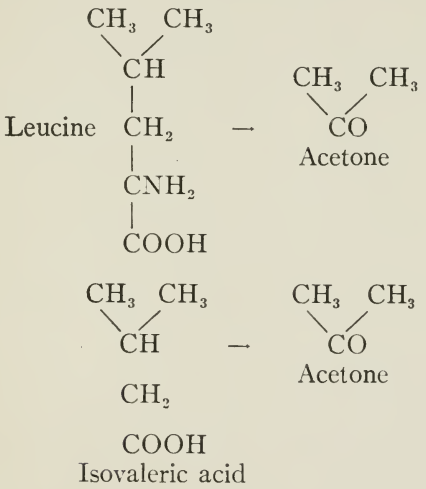
ing more of the enzymes participating in the process. That enzymes should be the cause of all combustion in the animal body, is a matter of great importance. There can be no doubt that the production of enzymes, and the control of their action, depend upon the living protoplasm. By the discovery of the metabolism-enzymes, one stage of the living process becomes a simple chemical process, and we are enabled to hope that further investigations will show that subsequent stages are susceptible of equally simple explanation.

We are, to-day, far from attaining this goal, but we can show that the surviving organ with its structure acts in another way than any extract of the same organ. For instance, extracts of the mucous membrane of the small intestine of fish contain erepsin; the extracts dissociate peptone into amino-acids, but leave these amino-acids untouched. But I have placed the surviving intestine of these same fish (Labrus or Crenilabrus) in Ringer's solution, filled the intestine with a solution of peptone or tyrosine, and allowed the intestine floating in the Ringer's solution to absorb the peptone solution and thus to transfer its contents into the Ringer's solution outside. In a high invertebrate, the Octopus, I found amino-acids, but in fish, during the passage through the wall, peptone and tyrosine are transformed. Ammonia is split off in great amount.[1]

A second example is the liver. If we allow liver extract

[1] O. Cohnheim: Zeitschr. f. physiolog. Chem., 59 (1909).

to act upon amino-acids, they are not at all changed; but if we use the undestroyed liver as a surviving organ, and cause a stream of blood to flow through the vessels, then leucine, isovaleric acid, and some other cleavage-products of proteins, when added to the blood, are converted into acetone, as has been pointed out by Embden.[1]



A third less evident example which can be cited against the theory of oxidation accomplished by enzymes, is the absorption of oxygen by the eggs of the sea-urchin, studied by Warburg.[2] The oxidation is influenced by the surface of the eggs.

The difficulties of obtaining and bringing zymase into

[1] G. Embden: Hofmeister's Beitr., 8 (1906).
[2] O. Warburg: Zeitschr. f. physiolog. Chem., 66 (1910).

solution was mentioned in the early lectures. Zymase can be precipitated together with the proteins of the press-juice, by acetone or by alcohol and ether, or it can be salted out like other enzymes. It is resistant to changes in reaction like living yeast, but suffers destruction in a short time whatever the reaction. It is more stable when allowed to act in the presence of sugar than in the isolated state, but even then loses activity within three to four days. Yeast, and therefore the press-juice of yeast, yields a proteolytic enzyme, the so-called endotrypsin or endotryptase. Buchner has not succeed in separating this enzyme, and suggests that zymase is destroyed by endotryptase, and that this is the reason for its instability. The action of the zymase is increased if we add sodium phosphate to the press-juice. Boiled press-juice or extracts of boiled yeast, which contain phosphates, likewise augment the action of zymase. It is not certain whether phosphates improve the action of the zymase by influencing the reaction or other conditions, or whether it functionates as a true co-ferment, like the manganese in the case of laccase, or the glycocholic and taurocholic acids in the case of pancreatic steapsin.

The qualities of the lactacidase and the alcohol-oxidase yielded by the bacteria that convert sugar into lactic acid, or alcohol into acetic acid, which have been studied by Buchner and Herzog,[1] resemble the qualities of zymase so far as difficulty of extraction and instability

[1] See J. Meisenheimer: Biochem. Zentralbl., 6 (1907).

are concerned. Plants of different classes contain, according to Hahn,[1] Palladin,[2] Stoklasa,[3] and other authors, enzymes which convert sugar, and these are called respiratory enzymes. Most of them form alcohol like zymase, but it seems that alcohol is not always formed, and then only when oxygen is lacking. The chemical processes in plants in the presence of oxygen are less understood. The old claims that yeast also ferments sugar only when there is a lack of oxygen, have been refuted by Buchner.

The glycolytic enzyme in the animal body seems to be even more unstable than the metabolism-enzymes of bacteria. Stoklasa thought he had found enzymes in the animal tissues which could convert sugar and form alcohol. There can be no doubt that he was deceived by experimental error. He did not observe tissue-enzymes, but bacteria grew in his solutions, which were not protected by antiseptic substances in sufficient quantity, and they developed carbon dioxide and formed alcohol. If we add glucose to muscle extract prepared by any process, no sugar is changed; but the sugar is attacked, as I have already stated, if we add to the muscle extract a certain substance—the co-ferment of the glycolytic enzyme of muscle, yielded by the pancreas. This glycolytic enzyme is highly sensitive to an acid reaction, and is destroyed, even in the frozen state, in one or two days, and in a few hours at the temperature of the room or body.

[1] M. Hahn: Ber. d. deutsch. chem. Ges., 33 (1900).
[2] W. Palladin: Zeitschr. f. physiolog. Chem., 55, 56 (1908).
[3] J. Stoklasa: *ibid.*, 50 (1906).

Because the muscle either does not contain a proteolytic enzyme, or contains a very weak one, the loss of power cannot be due to a destruction by a trypsin-like enzyme. The presence of oxygen is necessary or at least has a favorable influence upon the action of the glycolytic enzyme.

Vernon [1] has tried to throw light upon the chemical character of respiratory substances, that is, of enzymes, and he observed the gaseous exchange of surviving organs after the addition of poisons. He saw that hydrocyanic acid, some metals, hydroxylamin, and other substances which react easily with aldehydes, are poisons which check oxygen absorption, and he suggests that perhaps respiratory substances resemble aldehydes.

No other metabolism-enzymes have thus far been isolated from the tissues and cells, though it is a probable hypothesis that enzymes take part in all the chemical processes of the body. But if we accept this hypothetical view, we must assume the existence of the following metabolism-enzymes, though they have not as yet been found and only the actions to which they give rise are known. With a mental reservation, we may assume their existence from their actions:

1. Oxidases which completely burn fats, sugar, and proteins in the muscles and glands of the higher animals.

I have already stated that anoxybiotic processes, mere splittings, are incompatible with the active processes of

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[1] H. M. Vernon: Journal of Physiology, 39 (1909).

animal metabolism, and that fats and sugar must be completely oxidized. Nevertheless it is possible that oxidation is accomplished by several enzymes, working one after another, like the proteolytic enzymes of the alimentary canal. Buchner has suggested that zymase does not immediately form alcohol and carbon dioxide, but first forms lactic acid, and the lactic acid is then converted into alcohol and carbon dioxide by a second, separate enzyme. The proofs brought forward for this view do not seem to me to be conclusive. The suggestion is only supported by the observation of very small quantities of lactic acid besides the alcohol, and by the fact that this small quantity of lactic acid varies with individual yeasts. But lactic acid can also be formed from other products produced by the yeast, especially from alanine and protoplasmic proteins, and set free by the endotryptase occurring in all yeasts and in all preparations of zymase. In higher animals, Zuntz and his collaborators have observed that the absorption of oxygen and the output of carbon dioxide increases immediately when the work done by the muscles or glands increases, and that the respiratory quotient, the proportion CO_2/O_2, is not at all changed if work is done by muscles. Neither is oxygen stored up even for a few minutes, nor is carbon retained anywhere in the organism. If we are really dealing with a plurality of enzymes, these enzymes must work simultaneously; they cannot be separated in space or time.

2. Enzymes which split fats and amino-acids, and per-

haps sugars, at different stages, and thus form intermediate products which may have special functions in the organism, or may be used for building new material, or they may occur under abnormal conditions, when an enzyme or a function fails. We know now the following cases, but I wish to emphasize that the enzymotic nature of these processes is to-day a mere hypothesis. Experimental observations tell us, on the contrary, that all these processes are connected with the structure of the organs. They cannot be observed in extracts, but they can be observed only in the living organism or surviving organs.

1. Butyric acid is converted into β-oxybutyric acid in diabetes.

2. Fatty acids, as palmitic and stearic acids, are converted into β-oxybutyric acid and acetoacetic acid.[1] The conversion occurs in human diabetes, pancreatic diabetes, and in starvation, *i.e.*, when no sugar is burned. It occurs in the surviving liver. The conversion of β-oxybutyric acid into acetoacetic acid is a reversible process (see page 133).

3. Some cleavage-products of proteins, leucine, tyrosine, phenylalanine, phenyl-lactic acid, homogentisic acid, isovaleric acid, as well as butyric and oxybutyric acids, are converted into acetone. The conversion occurs in the liver.[2]

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[1] H. C. Geelmuyden: Skandinav. Arch. f. Physiol., 11 (1900).—A. Magnus-Levy: Arch. f. exper. Path. u. Pharmak., 42 (1899); 45 (1901).—J. Baer and L. Blum: *ibid.*, 55, 56 (1906).—Hofmeister's Beitr., 1 (1907).

[2] G. Embden: *ibid.*, 8 (1906).—L. Borchardt: *ibid.*, 9 (1907).

4. Glycerin is converted into glucose or glycogen.[1] The conversion occurs in diabetes.

5. Alanine, glutamic acid, and perhaps other cleavage-products of protein, are converted into sugar. The conversion occurs in diabetes.[2]

6. Glucose is converted into glycuronic acid:

$$\begin{array}{l} CH_2OH \\ \dot{C}HOH \\ \dot{C}HOH \\ \dot{C}HOH \\ \dot{C}HOH \\ \dot{C}OH \end{array} \quad = \quad \begin{array}{l} COOH \\ \dot{C}HOH \\ \dot{C}HOH \\ \dot{C}HOH \\ \dot{C}HOH \\ \dot{C}OH \end{array}$$

The conversion occurs when such substances like chloral, thymol, camphor, etc., enter the organism. They are conjugated with glycuronic acid in the liver.

7. Leucine, isoleucine, and valine, are, according to Felix Ehrlich,[3] converted into isoamyl alcohol, active isoamyl alcohol, and isobutyl alcohol.

The three alcohols form the fusel oil found in liquors manufactured by yeast-fermentation. Corresponding enzymes convert other cleavage-products of proteins into alcohols, which, coupled with other alcohols, form the odorous compounds of roses, apples, pears, and other flowers and fruits.

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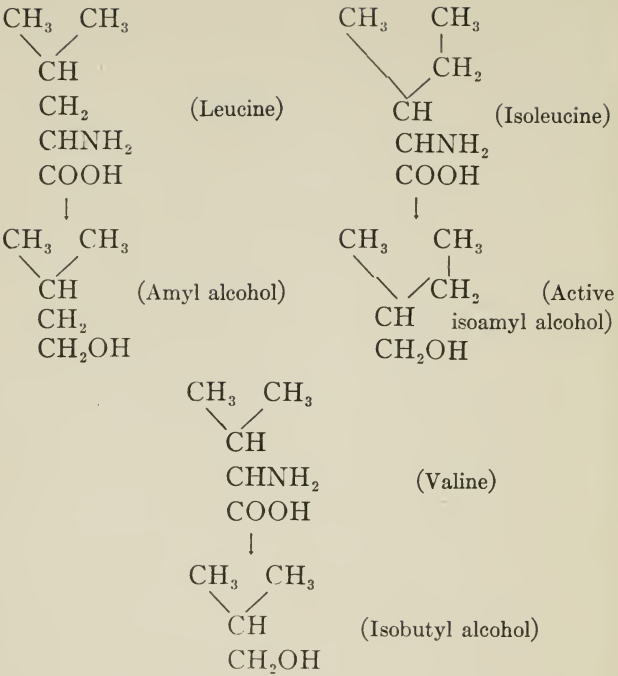
[1] M. Cremer: Münchener med. Woch., 1901.—H. Lüthje: Deutsch. Arch. f. klin. Med., 80 (1904).

[2] G. Lusk: American Journ. of Physiol., 25, 1909.

[3] F. Ehrlich: Biochem. Zeitschr., 2 (1906).—H. Thierfelder: Zeitschr. f. physiol. Chem., 11 (1887).



8. Amino-acids split off ammonia in passing through the intestinal wall of fish.[1]

These aminolytic enzymes seem to be widely distributed in both the vegetable and animal kingdoms, as well as in bacteria. The well-known products of putrefaction, skatol, phenylacetic acid, etc., are formed by aminolytic processes. These aminolytic enzymes do not set free energy, and it might be easier to isolate them than the metabolism-enzymes in the strict sense.

[1] O. Cohnheim: Zeitschr. f. physiol. Chem., 59, 61 (1909).

9. Ornithine and lysine are converted into putrescine and cadaverine.

$$\begin{array}{l} CH_2NH_2 \\ \dot{C}H_2 \\ \dot{C}H_2 \\ \dot{C}HNH_2 \\ \dot{C}OOH \end{array} = \begin{array}{l} CH_2NH_2 \\ \dot{C}H_2 \\ \dot{C}H_2 \\ \dot{C}H_2NH_2 \end{array} + CO_2$$

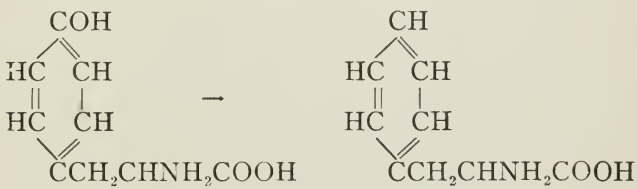
The conversion occurs through the action of putrefaction bacteria, and in human metabolism in cases of cystinuria.

10. Cysteine is converted into taurine.

$$\begin{array}{l} CH_2SH \\ \dot{C}HNH_3 + 3O = \\ \dot{C}OOH \end{array} \begin{array}{l} CH_2SO_2OH \\ \dot{C}H_2NH_2 \end{array} + CO_2$$

This conversion occurs in the liver.[1]

11. Tyrosine and phenylalanine are converted into homogentisic acid in the so-called alkaptonuria.[2],[3]

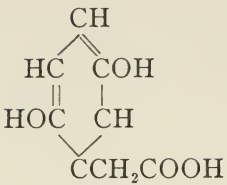


Tyrosine Phenylalanine

[1] G. v. Bergmann: Hofmeister's Beitr., 4 (1904).

[2] E. Baumann and v. Udransky: Zeitschr. f. physiolog. Chem., 13, 15 (1889).—A. Ellinger: *ibid.*, 29 (1900).

[3] E. Meyer: Deutsch. Arch. f. klin. Med., 70 (1901).



Homogentisic acid

12. Lactic acid is formed from an unknown compound in muscles. An enzyme widely distributed in the organism causes the acid reaction in tissues and organs immediately after death, or under special conditions during life. The reaction [1] of the blood, lymph, and tissues of animals, is neutral, and the organism is well provided with means to maintain the neutrality of its tissues. It is maintained (1) by the twofold character of carbonic acid, which can react as an acid with sodium and neutralize it, or can become CO_2, a neutral gas; (2) by the twofold character of proteins and all cleavage products of proteins, which are amphoteric electrolytes, and react both with bases and acids, and neutralize them to a certain extent; (3) by the presence of phosphates on the one hand, and of ammonia on the other; and (4) by burning all acids formed in the immediate metabolism into neutral carbon dioxide. But this combustion is an oxidation, and where oxygen is lacking, acid reaction occurs. Thus it occurs immediately after death. In warm-blooded animals, especially the larger, the high temperature of the body

[1] O. Cohnheim: "Physiologie der Verdauung u. Ernährung," Berlin, p. 347. L. Henderson: Ergebnisse der Physiologie, 8 (1909).

remains for a time after death, and the enzymes can then still act energetically. But oxidation is checked with the last heart-beat, and the tissues now become acid in reaction. We can observe this acid reaction in the liver, the pancreas, the kidney, the brain, and the muscles. If we kill dogs, cats, or rabbits in the laboratory, and study the organs a few minutes after death, we find that the reaction of the kidney and the brain may be acid, because these organs have the greatest metabolism, while the liver and the pancreas may still exhibit a neutral reaction. From the ox we can obtain the liver and pancreas only forty to sixty minutes after death, and in this time these glands become acid.

In muscles, an acid reaction produces rigor mortis. Kühne was the first to show that rigor mortis is the clotting of the fluid muscle-plasma, and that a change in the myosin is the cause of this clotting. Many authors have supposed that myosin is coagulated by a ferment, like fibrinogen by thrombin, or, as a common example, like casein by rennet. But the occurrence of rigor mortis is always connected with an acid reaction; myosin is precipitated like many other proteins, as the so-called globulins, by acid. By the precipitation, the fluid sarcoplasm becomes solid in the muscle-fibrils, and rigor mortis occurs. Later, the solid coagulum contracts, and squeezes out the fluid, just as the blood serum is squeezed from clotted blood and the rigor mortis is relaxed again. Thus the formation of acid in muscles is an important phenomenon, and investigators have tried to throw light upon the

process. The acid formed in muscle deprived of arterialized blood is lactic acid, and the formation of lactic acid under different conditions, the time of formation, the quantity of formation, etc., have been pointed out recently by Fletcher and Hopkins.[1] The chief result obtained by these authors is that the formation of lactic acid is closely connected with the life of the muscles, but only with life under abnormal conditions, especially with lack of oxygen. If sufficient oxygen is supplied, not only is no lactic acid formed, but even lactic acid which has previously developed is destroyed in the presence of oxygen. It might be suggested that a ferment, occurring in muscles, converts sugar, with a free supply of oxygen, into carbon dioxide; and with a lack of oxygen, into lactic acid. But this explanation is not in agreement with the results of Fletcher and Hopkins, that lactic acid is neither formed in dead muscles nor in muscle-extracts, but only in surviving muscles deprived of oxygen, and they emphasize that no evidence has been brought forward for sugar as the source of lactic acid. The formation of lactic acid does not occur in solution, and we meet here again with the phenomenon, that some reactions are limited to certain structures, and we hypothesize when we speak of an enzyme which gives rise to lactic acid when there is a lack of oxygen.

Lack of oxygen occurs after death, but it occurs also during life. Araki,[2] in Hoppe-Seyler's laboratory, demonstrated that with a lack of oxygen, dogs and rabbits

[1] W. M. Fletcher and F. G. Hopkins: Journ. of Physiology, 35 (1907).
[2] T. Araki: Zeitschr. f. physiolog. Chem., 15 (1891).

form and eliminate great quantities of lactic acid, and it is an old experiment, that the contracting frog's muscles become acid. If we inject litmus solution into a frog, cut one sciatic nerve and stimulate it, the stimulated leg becomes red, while the unstimulated leg shows a blue or violet color. It is necessary, however, to tie the leg, and prevent a free oxygen supply. According to Zuntz,[1] acids, probably lactic acid, are formed in hard muscular work which exceeds physiological limits.

The formation of lactic acid is one of the reasons which have provoked the suggestion of the anoxybiotic processes in tissues. I conclude only, that tissues yield an enzyme which, under special conditions, can form lactic acid. The lactic acid is the δ-α-oxypropionic acid, corresponding to alanine.

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[1] N. Zuntz and J. Geppert: Pflüger's Arch., 62 (1896).—A. Bornstein and E. Poler: *ibid.*, 95 (1903).

CHAPTER XXII

The Fibrin-Ferments

It remains but to mention an enzyme which differs from all enzymes described so far, the fibrin-ferment, which provokes the clotting of blood. In this clotting of the blood the chemical steps are known, but it was uncertain whether an enzyme provokes them. The enzymotic character of blood-clotting cannot be denied, and the chemical process is not explained.

Blood, when shed from the blood-vessels of a living body, is perfectly fluid. In a short time, however, it becomes viscid. The viscidity increases rapidly until the whole mass of blood becomes a complete jelly. The factors playing a rôle in the coagulation of blood are extremely complicated, there being four different substances which react upon one another. In the first place, the blood contains the soluble fibrinogen, which, as Hammarsten [1] has demonstrated, occurs in the circulating blood as a special albuminous body. Then there is the true fibrin-ferment, which, in all probability, is also present in circulating blood as zymogen.[2] The third substance is the activator [2] of the fibrin-ferment, or the thrombin, as it is called after activation.

[1] O. Hammarsten: Zeitschr. f. physiolog. Chem., 22 (1896); 30 (1899).

[2] P. Morawitz: Hofmeister's Beitr., 4 (1903); Deutsch. Arch. f. klin. Med., 79 (1903); Ergebnisse d. Physiologie, 4 (1905).

The activator has been designated as "thrombokinase." In order to effect the change induced by thrombokinase, a fourth factor, namely lime salts, is necessary.

The process by which fibrinogen is changed into fibrin is not known. All that is known is, that an insoluble substance is produced which has always been considered as being similar to casein which clots by the agency of rennet. But whether this process involves a cleavage, and whether one of the products of such cleavage is the insoluble substance referred to, is a question we are not prepared to answer. Nor can the relations be explained between the thrombin, or its zymogen, the lime salts, and thrombokinase, although it would seem that there exists a certain quantitative relation between the kinase and the thrombin similar to that of the activating process of trypsin in the cases already referred to. Lime salts, thrombin, and fibrinogen are present in the blood. The reason why circulating blood does not coagulate is that thrombokinase is absent. Thrombokinase, however, is present in the blood platelets, and the investigations of Deetjen[1] have rendered it probable that it exists nowhere else. The blood platelets which constitute the third component of the blood—the other two being the red and white blood corpuscles—are complete cells, just like the white corpuscles, provided with nucleus, protoplasm, and ferments. The fact that formerly so little was known of them is explained, first, by the fact that

[1] H. Deetjen. Virchow's Arch., 164; Zeitschr. f. physiolog. Chem., 63 (1909).

they are smaller than the leucocytes and red blood corpuscles, and second, that they are very easily destroyed and go into solution. Deetjen was the first to succeed in preserving the blood platelets outside the organism sufficiently long to examine thoroughly and fix them. He obtained cultures on agar-agar in the same way as bacteria are grown on the same substance; and, by adopting special precautions, he was able to observe them under the microscope.

The fact that these blood platelets are exceedingly sensitive to all kinds of influences, is intelligible from the function they have to perform, which is to make the blood coagulate. If the object of the blood coagulation, that is, the inhibition of hemorrhage, is to be attained, it is necessary for the blood to coagulate whenever it leaves the vessels. Therefore, the mere fact of blood flowing out under abnormal conditions must suffice to coagulate it, and for this reason the blood platelets, which contain the thrombokinase, are so exceedingly sensitive to all influences which are exerted upon the blood from without the normal vessels.

Deetjen has found two facts: (1) The blood platelets perish whenever they come in contact with a rough surface; and it is a well-established fact that blood coagulates not only outside the blood-vessels, but also within them whenever the normal smooth endothelium has been destroyed; and (2) the blood platelets invariably perish when the blood becomes alkaline in reaction. Fluids and tissues of the organism under physiologic

conditions always have a rather constant neutral reaction, and the organism has means to restore this reaction if any extraneous influence impairs it. (See page 158.) We have mentioned agents which prevent its turning acid. In the present instance, we have to consider agents which prevent its turning alkaline, because the blood platelets are destroyed by an alkaline reaction. Now, alkaline reaction may occur under two conditions, one of which is an artificial one. The glass utensils used for scientific experiments are not entirely insoluble in water; after a while they communicate to the water some alkalinity, and these small quantities of alkali, which exist also in distilled water stored in glass vessels, even in beakers, slides, and cover glasses, are sufficient to destroy the blood platelets. Deetjen has found that it is possible to observe the blood platelets properly only by discarding glass in the apparatus used, such as vessels, beakers, test tubes, slides, and cover glasses, and replacing it by quartz, which does not communicate alkali to water.

Furthermore, carbonic acid is contained in the blood, both as gas and combined with sodium. If blood comes into contact with the outside air, that part of the carbonic acid which is present as gas escapes; and the relation between the carbonic acid and the sodium is loosened by reason of the reduced carbonic acid; and if blood has been in contact with the extraneous air only for a short time, its reaction becomes alkaline. Even this slight alkalinity is sufficient to destroy the blood platelets, to

permit the occurrence of thrombokinase, and to cause coagulation of the blood.

The relations between thrombokinase and the protease which is found in the blood platelets (see p. 112) are not understood. According to Hammarsten, the conversion of fibrinogen into fibrin is a slight dissociation and it can be possible that the clotting of blood is a proteolytic process like the clotting of milk, and that the weak proteolytic enzyme of blood platelets is set free by this dissolution and acts as fibrin-ferment.

Fibrin-ferment together with blood coagulation is therefore one of the means of protection which the organism employs to ward off hostile interference, and, in connection herewith, we come to a special class of ferments. Fibrin-ferment has nothing to do with metabolism in the normal processes of the organism; it is merely a factor of safety with which the organism is provided. It will thus be seen in what effective manner the properties of the blood platelets have been made serviceable to insure the safety of the organism.

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